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**BISPHOSPHONATE TREATMENT IN CHILDREN
WITH OSTEOPENIA IMPERFECTA**

BENEFITS AND CONCERNS

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ACADEMIC DISSERTATION

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Abstract

Osteogenesis imperfecta is an inherited disorder of connective tissue characterized by bone fragility and low bone mass. It is caused by quantitatively or structurally abnormal type I collagen. Bisphosphonates, a group of anti-resorptive drugs, are commonly used as a medical therapy in children with osteogenesis imperfecta. A large number of studies have demonstrated their beneficial effects on bone mass and density. Much of their effects, especially long-term effects, in growing children remains unclear, however. This investigation studied the effects of bisphosphonate treatment on the development of cranial base pathology, dental development, and characteristics of femoral fractures. In addition, zoledronic acid, the most recent intravenous bisphosphonate, was evaluated in the treatment of children with osteogenesis imperfecta.

The patient population comprised children with osteogenesis imperfecta followed at the Metabolic Bone Clinic, Children's Hospital, Helsinki University Hospital, Finland. The treatment response of zoledronic acid was analyzed in 17 children with mild osteogenesis imperfecta. The treatment effectively increased bone mass and density, and a decreasing trend in fracture incidence was found. These results were comparable to pamidronate, the most studied intravenous bisphosphonate in children with osteogenesis imperfecta. However, zoledronic acid has the advantage of more convenient infusion protocol.

Cranial base pathology is one of the most severe complications of osteogenesis imperfecta. The longitudinal analysis of skull base morphology from lateral skull radiographs obtained in 22 children with osteogenesis imperfecta indicated that cranial base pathology may develop despite of bisphosphonate treatment. The analysis also suggested, however, that treatment started in infancy may delay the development of the pathology. Regarding the dental development, bisphosphonate treatment was found, in the evaluation of dental panoramic tomographs of 22 patients, to delay the development of the permanent dentition. However, since the children with osteogenesis imperfecta were found to be inherently advanced in terms of dental age and eruption of the permanent teeth, the treatment rather seemed to normalize the timing of dental development.

Atypical femoral fractures caused by bisphosphonate treatment have recently been a major concern in women with postmenopausal osteoporosis. The radiographic analysis of 127 femoral fractures occurred in 39 children with osteogenesis imperfecta showed that the fractures often represented atypical characteristics regardless of the patients' previous exposure to bisphosphonates. Furthermore, no changes in the location or configuration of the fractures were found in relation to bisphosphonate treatment. Instead, the characteristics reflected the severity of osteogenesis imperfecta.

In conclusion, bisphosphonate therapy can be considered as a reasonably effective and well tolerated treatment in children with osteogenesis imperfecta. Further studies are, however, needed to elucidate their long-term effects, and to optimize their treatment protocols.

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List of original publications

This thesis is based on the following publications:

- I Vuorimies I, Toiviainen-Salo S, Hero M, Mäkitie O (2011) Zoledronic acid treatment in children with osteogenesis imperfecta. *Horm Res Paediatr* 75: 346-353.
- II Arponen H, Vuorimies I, Haukka J, Valta H, Waltimo-Sirén J, Mäkitie O (2015) Cranial base pathology in pediatric osteogenesis imperfecta patients treated with bisphosphonates. *J Neurosurg Pediatr* 15: 313-320
- III Vuorimies I, Mäyränpää MK, Valta H, Kröger H, Toiviainen-Salo S, Mäkitie O (2017) Bisphosphonate Treatment and the Characteristics of Femoral Fractures in Children with Osteogenesis Imperfecta. *J Clin Endocrinol Metab* 102: 1333-1339.
- IV Vuorimies I, Arponen H, Valta H, Tiesalo O, Ekholm M, Ranta H, Evälahti M, Mäkitie O, Waltimo-Sirén J (2016) Timing of dental development in osteogenesis imperfecta patients with and without bisphosphonate treatment. *Bone* 94: 29-33

The publications are referred to in the text by their above Roman numerals. These articles were reprinted with the permission of their copyright holders.

Abbreviations

AFF	Atypical femoral fracture
ALP	Alkaline phosphatase
ATP	Adenosine-5'-triphosphate
BP	Bisphosphonate
BMC	Bone mineral content
BMD	Bone mineral density
aBMD	Areal bone mineral density
vBMD	volumetric bone mineral density
CRTAP	Cartilage-associated protein
CT	Computed tomography
CTx	Collagen type I C-terminal telopeptide in urine
CyPB	Cyclophilin B
DPT	Dental panoramic tomograph
DXA	Dual-energy X-ray absorptiometry
FKBP65	FK506 binding protein 65
HSP47	Heat shock protein 47
ICTP	Collagen type I C-terminal telopeptide
INTP	Collagen type I N-terminal telopeptide
M-CSF	Macrophage-colony stimulating factor
MRI	Magnetic resonance imaging
NTx	Collagen type I N-terminal telopeptide in urine
OI	Osteogenesis imperfecta
OPG	osteoprotegerin
pQCT	Peripheral quantitative computed tomography
P3H1	Prolyl 3-hydroxylase 1
PICP	Procollagen type I C-terminal propeptide
PINP	Procollagen type I N-terminal propeptide
PTH	Parathyroid hormone
RANKL	Receptor activator of nuclear factor kappa B ligand

1 Introduction

Osteogenesis imperfecta (OI) is an inherited disorder characterized by bone fragility, skeletal deformities, and substantial growth deficiency. The clinical severity varies widely, ranging from mild forms without fractures to intrauterine fractures and perinatal lethality. Many other organ systems can also be involved. Typical extraskkeletal features are bluish discoloration of the sclerae and dentinal abnormality, often referred to as type 1 dentinogenesis imperfecta. However, these extraskkeletal features are also absent in many patients. (Rauch and Glorieux, 2004) The worldwide incidence of OI is approximately 1/15,000 to 1/20,000 births (Forlino and Marini, 2016).

OI is a disorder of quantitatively or structurally abnormal type I collagen, the most abundant protein in bone. In the great majority of the individuals with OI, the disease-causing mutation resides in one of the two genes encoding type I collagen (*COL1A1*, *COL1A2*). Recently, mutations in several other genes have been identified to cause OI as well. These genes code for proteins responsible for post-translational modification, folding, or secretion of type I collagen. (Forlino and Marini, 2016)

Before the time of medical therapies, the treatment of children with OI comprised of physical therapy, rehabilitation and surgical management. The goal was basically the same as today: taking care of muscle condition, maximizing functional capabilities, and preventing fractures. Surgical orthopedic interventions included correction osteotomies and use of intramedullary telescopic roding to correct long-bone deformities and prevent fractures. The immobilization after surgery or fracture was limited to the time strictly necessary for healing. These non-medical treatments still remain the mainstay of treatment in children with OI, albeit the introduction of bisphosphonates has had a revolutionizing impact on it. (Antoniazzi *et al.*, 2000)

Bisphosphonate (BP) therapy is today by far the most used medical treatment modality in children with OI. A large number of studies, mainly performed with intravenous pamidronate, have shown their beneficial impact on bone mass and mineral density (Glorieux *et al.*, 1998; Rauch *et al.*, 2003). Many of these studies have also suggested

improvement in clinical outcomes, such as fracture rate, mobility, and quality of life (Astrom and Soderhall, 2002; Lindahl *et al.*, 2016). However, the question about clinical benefits still remains controversial.

As anti-resorptive agents, BPs reduce the remodeling activity in bone. This has been speculated to cause accumulation of microcracks, potentially leading to stress fractures in long-term use. Bone resorption also plays an important role in many developmental processes in a growing child, such as development and eruption of the dentition, and some concerns remain whether BP treatment would have a detrimental effect on these processes. The present study was undertaken to evaluate the benefits and long-term safety of BP treatment in the treatment of children with OI.

2 Review of the literature

2.1 Bone

2.1.1 Structure

Bones can be divided in three categories according to the shape: long bones are located in the shafts of limbs; short bones are cuboidal in shape and located in wrists and ankles; flat bones, such as sternum, ileum, and skull, protect inner organs. Some bones, such as facial bones, do not fall within any of these categories and are often called irregular bones. Furthermore, macroscopically bone can be divided into compact (*i.e.* cortical) bone and trabecular (*i.e.* cancellous or spongy) bone. All bones have a superficial layer of compact bone that provides the strength for weight bearing. The more capacity for weight bearing is needed, the thicker this layer is. Hence, the layer of compact bone is thickest near the middle of the shafts (diaphysis) of lower-extremity long bones. Trabecular bone forms the central mass of all bones and is the metabolically active part of the skeleton. Bone marrow is located within the medullary cavity between the trabeculae of the trabecular bone. (Clarke, 2008)

2.1.2 Bone cells

Osteoblasts

Osteoblasts are bone-forming cells which derive from mesenchymal progenitor cells, commonly referred to as mesenchymal stem cells. The active osteoblasts form initial unmineralized bone, called osteoid, by secreting type I collagen and other bone matrix proteins. Among these bone matrix proteins are alkaline phosphatase (ALP) and osteocalcin, used clinically as markers of osteoblast activity/bone-forming activity. Subsequently, the osteoblasts initialize and regulate mineralization of the osteoid by secreting vesicles that concentrate calcium and phosphate. At the completion of bone formation, approximately 50 to 70% of the osteoblasts undergo apoptosis, while the rest differentiate to bone-lining cells and osteocytes. (Clarke, 2008; Dirckx *et al.*, 2013)

Osteocytes

By constant secretion, osteoblasts surround themselves with osteoid and gradually isolate themselves from the adjacent cells. Of the osteoblasts embedded in the bone matrix, those that do not undergo apoptosis further differentiate to osteocytes, the main cells of already formed bone. (Dirckx *et al.*, 2013) Although surrounded by osteoid, osteocytes connect to adjacent osteocytes by long cytoplasmic processes and form a network-like structure, serving as mechanotransduction network, by which they sense mechanical loading to optimize the relation of bone formation and resorption according to the need (Bonewald, 2011). They are also responsible for maintaining the bone matrix, and are even capable of synthesizing new matrix (Zamboni Zallone *et al.*, 1983). Osteocytes also have a limited capability to degrade the bone matrix, and this in its turn is thought to be related to calcium homeostasis (Wysolmerski, 2013).

Osteoclasts

Osteoclasts, the cells responsible for bone resorption, originate from bone marrow and are members of monocyte/macrophage family. Two cytokines crucial to osteoclastogenesis are receptor activator of nuclear factor kappa B ligand (RANKL) and macrophage-colony stimulating factor (M-CSF). Osteoprotegerin (OPG) in turn lowers bone resorption activity by inhibiting RANKL. (Clarke, 2008) Osteoclasts are multinucleated and polarized cells that have extensively folded surface (ruffled border) against the bone supposed to be resorbed. The cell membrane of the ruffled border contains a large number of proton pumps that form an acidic microenvironment (pH values of up to 3 or less). The acidified milieu dissolves the mineral component of bone, and organic matrix, mainly type I collagen, becomes exposed and subsequently degraded by cathepsin K and metalloproteinases. The organic and inorganic degradation products are then endocytosed from the ruffled border membrane and transcytosed through the osteoclast to the extracellular space outside the basolateral surface. (Detsch and Boccaccini, 2015; Salo *et al.*, 1997)

2.1.3 Bone matrix and type I collagen

Majority of the bone mass consists of extracellular matrix, called bone matrix. It is mainly composed of minerals (50 to 70% of the weight), organic matrix (20 to 40%), water (5 to 10%), and a small amount of lipids. The mineral portion is almost completely comprised of crystallized hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ that provides hardness property, important to withstand mechanical loads. In addition to hydroxyapatite, bone matrix also contains carbonate, magnesium and acid phosphate serving as a source for calcium, magnesium and phosphate homeostasis. (Boskey, 2013)

The most abundant protein in bone matrix is type I collagen constituting approximately 90% of the organic phase of bone matrix (Boskey, 2013). Type I procollagen is a large structural protein consisting of two $\alpha 1(\text{I})$ polypeptide and one $\alpha 2(\text{I})$ polypeptide chain, which fold to a triple-helical formation. The triple-helical structure is essential for its function and requires a glycine residue at every third position of the chains. The complex folding process is multistage and requires several chaperon proteins. After folding, the procollagen molecule is secreted out of the cell, and the propeptides at the N- and C-termini (PINP and PICP) are cleaved off (Figure 1). Now, the protein is called type I collagen and it spontaneously polymerizes and forms fibrils. (Forlino and Marini, 2016) The functions of type I collagen include providing elasticity to bone, stabilizing bone matrix, supporting initial mineral deposition and binding other macromolecules. The noncollagenous protein content of bone matrix comprises a wide variety of proteins having a number of different functions. Among these functions are organization of bone matrix, coordination of cell-matrix and mineral-matrix interactions, metabolism, and regulation of mineralization process. (Boskey, 2013)

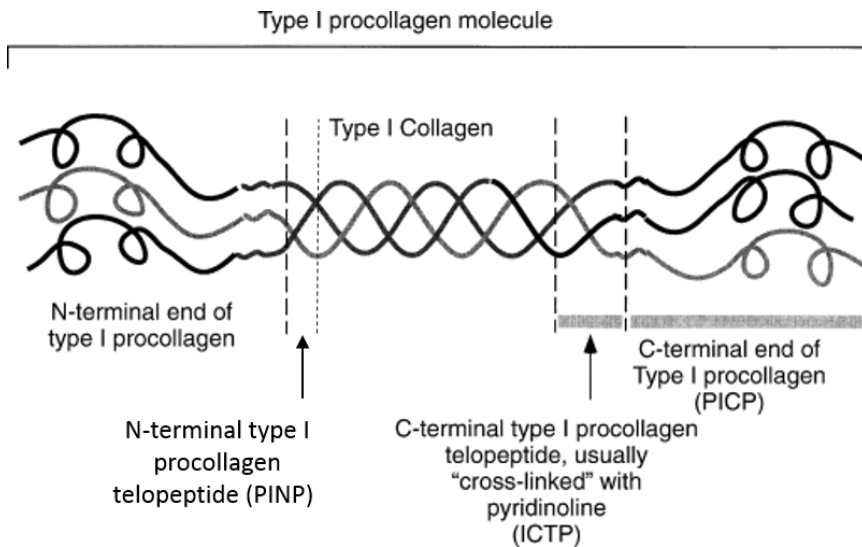


Figure 1. Type I procollagen molecule. The N- and C-terminal ends are cleaved off extracellularly, and can be used as biomarkers for bone turnover (modified from Urena & De Vernejoul, 1999).

2.1.4 Bone growth and turnover

Bone can be formed two ways: The bones of the extremities and weight-bearing axial skeleton develop with cartilage precursor (endochondral ossification). Flat bones, such as most of the bones of the skull, are in turn formed directly in mesenchymal condensations without any cartilage precursors (intramembranous ossification). The latter process is similar to periosteal bone formation that also takes place on the endochondral bones. The bones of the cranial base are an exception to the other bone of the skull, and form through endochondral ossification. After ossification of the rest of the long bones in the early childhood, special regions of cartilage remain between epiphyseal and metaphyseal bone at the ends of the developing long bone. These regions, called epiphyseal growth plates, are responsible for longitudinal growth, and retain their ability to grow new bone until the adult height is reached. The proliferating cells in the growth plates are chondrocytes that exceptionally start to produce a large amount of type I collagen. The matrix then calcifies which leads to degeneration and apoptosis of the chondrocytes. At the metaphysis, the newly formed calcified cartilage is resorbed by mononuclear cells of undetermined origin,

and osteoblasts replace it with new bone (primary trabecular bone). The new bone is further reorganized by osteoblasts and osteoclasts to respond the accommodation and physical requirements of the bone. All these events happen in their own differentiated zones, thus pushing epiphysis away from the diaphysis (Figure 2). (Berendsen and Olsen, 2015; Rauch, 2005)

Throughout the whole life, bone undergoes constant renewal and adaptation. This can occur in two ways: by modeling and by remodelling (Figure 2). Modeling is characteristic of growing bone, although it occurs to a lesser extent also in adults. In modelling, the bone adapts to mechanical forces by altering its mass, size, and shape. It is a process where bone forming osteoblasts and bone resorbing osteoclasts act on different surfaces of the bone and are not necessarily coupled. The net effect is often increase of cortical thickness and bone mass. The purpose of remodeling is not to alter bone features like with modeling, but rather to maintain bone strength and mineral homeostasis. The remodelling process resorbs old bone and forms new bone to replace it, hence preventing accumulation of micro fractures. The group of osteoclasts and osteoblasts acting together form a remodeling unit or basic multicellular unit. The cells of these units are in constant interaction, keeping the difference between removed and added bone (remodeling balance) close to zero in young adults. A decade or two after attainment of peak bone mass, the bone formation rate, however, fails to keep in pace with bone resorption, and bone loss begins. As opposed to modeling, remodeling occurs much more frequently in trabecular bone than in cortical bone; 80% of bone remodeling takes place in cancellous bone that comprises only 20% of the whole bone. (Clarke, 2008; Langdahl *et al.*, 2016)

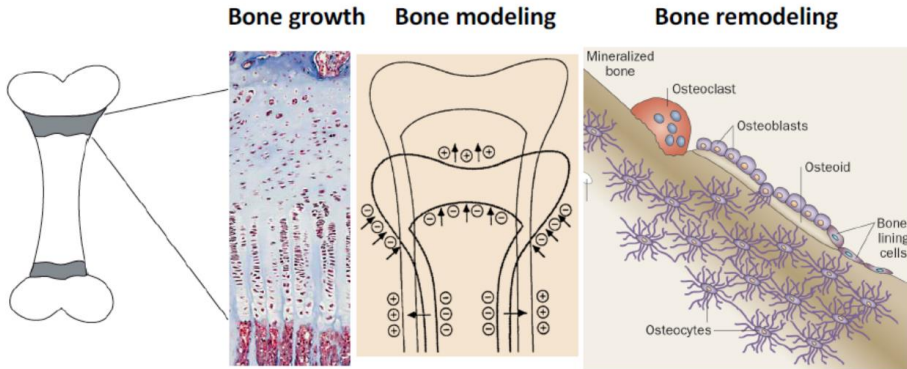


Figure 2. Bone growth and turnover. The longitudinal growth of long bones takes place in epiphyseal growth plates, where different phases of bone growth take place in five distinct zones: zone of reserve, zone of proliferation, zone of maturation and hypertrophy, zone of calcification, and zone of ossification. The shape of the bone is then altered in a process called modelling. Remodeling is a process, where old bone is replaced by new one.

2.1.5 Methods to assess bone and its metabolism

Reflecting the wide variety of functions that bone has, multiple different methods have been created to assess it. Radiological methods are mostly used for assessing the mass and structure, whereas biological methods measure mostly mineral metabolism and activity of bone cells. Bone biopsies can give us a specific view of bone at a histological level. Because of their invasive nature, they are, however, mostly used only in scientific research and rarely obtained in every day work in clinics.

Biochemical markers

Throughout the life, bone undergoes constant remodelling, to maintain structural quality. Specific molecules, bone markers, can be used to assess the intensity of this process. These markers can be measured in blood or urine. ALP and osteocalcin reflect the bone formation activity (Hlaing and Compston, 2014). PINP and PICP also are bone formation markers, but as cleavage products of type I procollagen, they also reflect the production of type I procollagen outside bone (Hlaing and Compston, 2014; Risteli and Risteli, 1993). Regarding bone resorption, the most used markers are the N-terminal and C-terminal type I collagen telopeptides (INTP and ICTP, respectively), which are small peripheral fragments of type I collagen degraded by osteoclasts (Figure 1). ICTP can be measured in blood or urine, but INTP only in urine. When measured in urine, ICTP is called CTx, and INTP NTx. All these bone markers reflect the intensity of whole remodelling process and can't be used to assess the remodeling balance (Szulc *et al.*, 2000). In contrast to adults, in growing children, both skeletal growth and high bone turnover rate elevate the bone markers (Huang *et al.*, 2011). Since the normative data is, however, currently limited, the bone markers are mainly used in longitudinal follow-up and assessing responses to medical treatments.

Radiological methods

The most used radiological method to evaluate the bone mineral density (BMD) is dual-energy X-ray absorptiometry (DXA). In DXA, the bone mineral content (BMC) is assessed within a selected bone area of interest by detecting the attenuation of two photon beams with different energies. Areal bone mineral density (aBMD) is then calculated by dividing BMC by the area, reported in g/cm². Hence, DXA does not adequately take into account the architectural structure and shape of the bone, underestimating bone mineral density, especially in children with short stature (Gafni and Baron, 2004). However, because of its widespread availability, high reproducibility, low radiation exposure, and relative affordability, it has established its role as a method of choice in diagnostics of osteoporosis. In children, the BMD values are compared with comprehensive database of age- and sex-matched controls and the results are reported as S.D. scores (z-scores) (Gordon *et al.*, 2008). The recommended sites for measurements are posterior-anterior lumbar spine, which mostly consists of trabecular bone, and whole body less head, which

reflects mostly cortical bone. In children, the hip is a less reliable site of measurement because of variability in positioning and difficulties in identifying bony landmarks (Golden and Abrams, 2014). Possible significant scoliosis and metal implants can impair the accuracy of measurements (Estrada *et al.*, 2014).

Another non-invasive method to assess bone density and fracture risk is peripheral quantitative computed tomography (pQCT), in which the measurement sites are distal tibia and/or distal radius (Binkley and Specker, 2000). As an advantage over DXA, it provides volumetric BMD, as well as information about bone geometry and strength. Currently, pQCT is, however, mainly used in research, while the use of different imaging protocols and the lack of reference values in children limit its use in clinical practice (Fonseca *et al.*, 2013). Bone quantitative ultrasonography (QUS) is a safe, fast and easy to use method to evaluate bone tissue, and is free of ionizing radiation. The most used sites for measurement are heel and phalanges. In addition to mineral density, it measures connectivity, elasticity and micro-architecture providing a measure of bone quality. However, due to a variability of devices and lack of standardization, the method QUS is not recommended in clinical practice for diagnosis of pediatric osteoporosis (Wang *et al.*, 2014; Pezzutti *et al.*, 2017). Despite the new methods in the evaluation of bone, conventional radiography has still maintained its role as gold standard. It is used to assess skeletal features of bone dysplasias as well as focal abnormalities. Furthermore, almost all fractures are detected and followed up with conventional radiographs, magnetic resonance imaging and computed tomography, primarily serving as secondary methods to assess occult and complex fractures. Conventional radiography also plays an important role in the evaluation of vertebral fractures, where the diagnostic accuracy of current DXA images is found to be insufficient (Mayranpaa *et al.*, 2007).

2.2 Teeth and development of dentition

Teeth, after completed formation are considerably stable organs with scarce regeneration mechanisms. Their most abundant structural tissue is dentin, being covered by enamel in the crown and by cementum in the root. Dentin is bone-like tissue, produced by odontoblasts that functions very similarly to osteoblasts. Contrary to bone, no osteocyte-

like cells exist in dentin. Instead, a single layer of odontoblasts remains lying on the surface of the dental pulp with long cell processes embedded in the dentinal tubules, and are responsible for the maintenance of dentin. However, their regeneration capability is limited and no remodeling occurs in dentin. (Bleicher, 2014) Cementum, a thin layer covering the roots of the teeth, also is bone-like tissue that is produced by osteoblast-like cementoblasts and maintained by osteocyte-like cementocytes. It is, however, structurally soft, and its main function is to serve, together with the principal fibers of periodontal ligament, as a connective layer between the dentin and the surrounding alveolar bone. (Yamamoto *et al.*, 2010) Enamel, though being the hardest tissue in the human body and consisting from 96% of hydroxyapatite crystals, is not connective tissue. It is instead mineralized material derived from ameloblasts, epithelial cells that degenerate at the time of tooth eruption. Hence, matured enamel is incapable of becoming regenerated. (Varga *et al.*, 2015)

Humans have two separate dentitions. The primary dentition, deciduous teeth, comprises 20 teeth and emerges usually between the first 6 months and 3 years of life. Typically, between the ages of 6 and 13 years, the deciduous teeth are lost, and 32 larger and more durable permanent teeth emerge, these are called the permanent dentition (Sperber *et al.*, 2001) (Table 1). The development of teeth starts during the fetal period when the oral epithelium and the underlying neural-crest-derived mesenchyme form the tooth bud. After the complex course of histological and morphological differentiation, the tooth bud develops into so-called bell stage. During this process, enamel knot, located at the tip of the epithelial bud, serves as a signaling center regulating the morphogenesis of the developing tooth (Thesleff *et al.*, 2001). In the bell stage, the mesenchymal cells are developed into dentin-secreting odontoblasts and the surrounding epithelium into enamel-secreting ameloblasts in the area preceding crown. (Caton and Tucker, 2009) The mesenchyme surrounding the tooth forms dental follicle, a loose connective tissue sac that gives rise to cementoblasts and periodontal ligament (Honda *et al.*, 2010). The calcification of the deciduous tooth germs commences already prenatally and is completed shortly after it. (Kjaer, 2014) Permanent teeth develop in a same manner, but budding from the invaginating dental epithelium of the primary teeth. The mineralization of the first permanent molars may start prenatally, but for the most part, it occurs after birth. The

permanent teeth normally erupt when their root length has reached three quarters of the expected length. First permanent molars and maxillary central incisors make an exception, often erupting with half-way developed roots. Another exception is made by the maxillary canines that do not erupt until their roots have obtained full length (Haavikko, 1970). The typical ages for the eruption of permanent teeth are shown in Table 1.

The mechanisms of tooth eruption still remain largely unclear. The crucial role of osteoblasts and osteoclasts in the eruption pathway is, however, evident. Osteoblasts form new alveolar bone at the base of the tooth, whereas osteoclasts resorb bone apical to the tooth, thus producing together an intra-osseous movement force towards the oral cavity (Wise *et al.*, 2011). The dental follicle also has a decisive role in this process, inducing and regulating the cells (Marks and Cahill, 1984). The induction of the whole event seems to be genetically programmed and not affected by external factors. In fact, the reference values of the eruption schedule dating back to 1933 by Logan and Kronfeld (Logan and Kronfeld, 1933) is still accurate, while growth curves and timing of the puberty have shifted drastically. The mechanisms of eruption do not differ between deciduous and permanent teeth except that with permanent teeth the overlaying root of the deciduous tooth has to be resorbed.

Table 1: Timing of dental development and eruption of teeth. (Sperber *et al.*, 2001) The time when the eruption of permanent teeth less 3rd molars commences is reviewed according to Finnish norms (Virtanen *et al.*, 1994).

Tooth	Tooth germ completed	Calcification commences	Crown completed	Eruption commences	Root completed
Deciduous					
Incisors	12-16 wk pc	3-4 mo pc	2-4 mo	6-8 mo	1.5-2 yr
Canines	12-16 wk pc	5 mo pc	9 mo	16-20 mo	2.5-3 yr
1st molars	12-16 wk pc	5 mo pc	6 mo	12-15 mo	2-2.5 yr
2nd molars	12-16 wk pc	6-7 mo pc	11-12 mo	20-30 mo	3 yr
Permanent					
Central incisors	30 wk pc	3-4 mo	4-5 yr	Max 7-8 yr/ Mand 6-8 yr	9-10 yr
Lateral Incisors	32 wk pc	Max 10-12 mo/ Mand 3-4 mo	4-5 yr	Max 7-10 yr/ Mand 7-9 yr	10-11 yr
Canines	30 wk pc	4-5 mo	6-7 yr	Max 10-13 yr/ Mand 9-12 yr	12-15 yr
1st premolars	30 wk pc	1.5-2 yr	5-6 yr	10-13 yr	12-14 yr
2nd premolars	31 wk pc	2-2.5 yr	6-7 yr	10-14 yr	12-14 yr
1st molars	24 wk pc	Birth	3-5 yr	6-8 yr	9-10 yr
2nd molars	6 mo	2.5-3 yr	7-8 yr	11-14 yr	14-16 yr
3rd molars	6 yr	7-10 yr	12-16 yr	17-21 yr	18-25 yr
pc=post conception, wk=weeks, mo=months, yr=years, Max=maxilla, Mand=mandible					

2.3 Osteogenesis imperfecta

Osteogenesis imperfecta (OI), also known as brittle bone disease, is an inherited disorder characterized by bone fragility and an insufficient amount of bone, causing increased fracture incidence and bone deformities. The earliest mention of OI-like patients in the literature date back to 1788 when Swede Olof Jakob Ekman described in his thesis a condition resembling OI in a family where three generations were affected (Ekman 1788). He named the condition “osteomalacia congenita”. The current term “osteogenesis imperfecta” has been used in the medical literature since 1840s (Baljet 2002).

2.3.1 Genetics and pathophysiology

Approximately 90% of the OI patients have so-called classical form of disorder that is dominantly inherited. The classical form is caused by a mutation in one of the two genes, *COL1A1* and *COL1A2*, encoding either of the two α -chains of type I collagen, the main organic component of the bone and dentin. Over 1,500 different mutations have been reported (Forlino *et al.*, 2011). The mild cases are usually caused by premature termination codons, leading to degradation of the protein in a process called nonsense-mediated decay. However, since the other allele is intact, osteoblasts still secrete intact type I collagen, the amount of which is just reduced to approximately half. (Willing *et al.*, 1996) In more severe cases, the mutation usually causes substitution of one of the glycine residues (80%), or splicing defect resulting in an in-frame deletion of a section of the chain (20%). The glycine residue is repeated as every third amino acid almost throughout the α -chain, which is essential for the formation of the triple-helical structure of collagen. These mutations ultimately lead to structurally abnormal and thus functionally impaired protein, albeit of normal quantity. (Forlino *et al.*, 2011)

In 2-5 % of the OI patients, the disorder is inherited recessively. In the last 15 years, an increasing number of mutations in different protein-coding genes have been reported to cause OI (Table 2). These mutations usually affect proteins that participate in post-translational modification, folding, or secretion of type I collagen. The mechanism of

action is best described for the proteins coded by *CRTAP*, *LEPRE1*, *PPIB*, *SERPINH1*, and *FKBP10*. Cartilage-associated protein (CRTAP), prolyl 3-hydroxylase 1 (P3H1), and cyclophilin B (CyPB) form together the collagen prolyl 3-hydroxylation complex that modifies collagen in the endoplasmic reticulum; the collagen prolyl 3-hydroxylation complex is required for its proper folding. Heat shock protein 47 (HSP47) and FK506 binding protein 65 (FKBP65) assist in turn in the formation and secretion of the procollagen triple helix. Impaired function of these proteins leads to recessively inherited OI with a moderate to severe phenotype. (Forlino *et al.*, 2011; Forlino and Marini, 2016) A mutation in the gene *IFITM5* that codes for interferon-induced transmembrane protein 5 has, on the other hand, been identified as a causative factor behind dominantly inherited OI and accounting for approximately 5% of OI cases. The mutation causes moderately severe OI with unique clinical features (Cho *et al.*, 2012; Semler *et al.*, 2012), described later in the section defining the classification of OI.

Since the genetic defects causing OI affect type I collagen protein, the main organic component of bone, it is not surprising that the bone material quality of patients with OI is decreased. Proper hydroxyapatite crystallization requires attachment to type I collagen protein. In OI, the hydroxyapatite crystals are smaller but more abundant than normally that leads to hypermineralization of the bone (Traub *et al.*, 1994). The abnormally high matrix mineralization in conjunction with disorganization of the crystals is suggested to be a major contributor to the stiffness and hardness of bone in OI (Fratzl *et al.*, 1996). In addition to the impaired bone material quality, another considerable contributor to bone fragility in OI is low bone mass. Histomorphometric studies of iliac crest biopsies of patients with OI have shown markedly lower cortical width and diminished external bone size, suggesting a defect in cortical bone modeling (Ste-Marie *et al.*, 1984; Rauch *et al.*, 2000). Moreover, OI is also characterized by a low amount of cancellous bone as a result of low trabecular number and thickness. The bone remodeling is found to be accelerated. The increase in number of osteoblasts outweighs their functional defect, thus leading to increased bone formation. Since the osteoclast activity is, however, increased as well, no net gain in bone mass occurs. (Rauch *et al.*, 2000)

Table 2. Different genetic defects causing OI. AD, autosomal dominant; AR, autosomal recessive. Type refers to clinical type of OI. (van Dijk and Silence, 2014; Bonafe *et al.*, 2015)

Gene	Protein product	Defect	Inheritance	Type
<i>COL1A1</i>	Collagen alpha-1 (I) chain	collagen 1 quantity or structure	AD	1,2,3,4
<i>COL1A2</i>	Collagen alpha-2 (I) chain	collagen 1 quantity or structure	AD	1,2,3,4
<i>IFITM5</i>	Interferon-induced transmembrane protein 5	Matrix mineralization	AD	5
<i>SERPINF1</i>	Pigment-epithelium-derived factor	Matrix mineralization	AR	3,4
<i>CRTAP</i>	Cartilage-associated protein	Collagen prolyl 3-hydroxylation	AR	2,3,4
<i>LEPRE1</i>	Prolyl 3-Hydroxylase 1	Collagen prolyl 3-hydroxylation	AR	2,3
<i>PPIB</i>	Cyclophilin B	Collagen prolyl 3-hydroxylation	AR	2,3,4
<i>SERPINH1</i>	Heat shock protein 47	Collagen chaperoning	AR	3
<i>FKBP10</i>	FK506 binding protein 65	Telopeptide hydroxylation	AR	3,4
<i>SP7</i>	Osterix	Osteoblast development	AR	3,4
<i>BMP1</i>	Bone morphogenic protein 1	Collagen processing	AR	3
<i>TMEM38B</i>	Trimeric intracellular cation channel B	Osteoblast development	AR	3
<i>WNT1</i>	Wingless-type MMTV integration site family, member 1	Osteoblast development	AR	3,4
<i>CREB3L1</i>	Old astrocyte specifically induced substance (Oasis)	COL1A1 transcription	AR	3
<i>PLOD2</i>	Procollagen lysyl hydroxylase 2	Telopeptide hydroxylation	AR	3

2.3.3 Clinical features and non-medical treatment

The incidence of OI is about 1/15,000-20,000 births worldwide (Forlino and Marini, 2016). Kuurila and colleagues suggested six per 100,000 individuals to be affected with disorder in Finland (Kuurila *et al.*, 2002).

The main feature of OI is bone fragility that causes increased fracture incidence throughout the life, and in severe cases, even prenatally. The fracture incidence markedly varies according to the severity of disease, patients representing the mildest end of the spectrum having not even a single fracture during their life, and patients representing the severest end suffering multiple major fractures annually (Rauch and Glorieux, 2004). The fracture incidence also varies by age. It commonly drops substantially after growth spurt, and rises again in women after the menopause in the adulthood when bone mass hormonally begins to decrease (Paterson CR *et al.*, 1984). The fractures can occur due to very minor traumas, for example with infants by normal handling. The care givers often undergo significant distress and sometimes the situation can even lead to over-caution and interfere with proper muscular development. Hence, valid information for the family lies at the core of the overall management of a newborn child with OI (Bozkurt *et al.*, 2014). In patients with severe OI, frequent long-bone fractures together with impaired bone mass leads to bending of the long bones. These deformities often lead to physical limitations, and the patients commonly need to use mobility aids, such as wheel chair and wheeled walker. Physiotherapy and rehabilitation hold true as a mainstay of treatment of children with OI improving markedly mobility and well-being (Binder H *et al.*, 1993). Correction osteotomies and intramedullary roding is used to straighten the bended femora and tibiae, typically, and to maintain the mobility. Scoliosis and kyphosis of the thoracolumbar spine, due to multiple vertebral compression fractures, are also common in OI. A thoracolumbar kyphoscoliosis, in conjunction with rib fractures and muscle weakness, may even progress to a level that leads to respiratory insufficiency and cor pulmonale, which have been the leading cause of death in adults with OI (Widmann *et al.*, 1999).

The most prevalent secondary feature of OI is short stature, though the magnitude varies markedly according to the severity of the disease. Most of the patients with mild or moderate OI have normal birth length, but those with severe OI, exhibit retarded growth already prenatally. The growth rate of patients with moderate to severe OI is greatly reduced, adult patients with moderate OI standing in a height of early teenager, and with severe OI, in a height of prepubertal child. Patients with mild OI grow parallel to normal grow curve and have normal adult height or slightly shorter. (Vetter *et al.*, 1992) The reason for shorter stature is unclear. Vertebral compression fractures, scoliosis, and long bone bending contribute to short standing height but they do not explain the deficient growth. It has been speculated whether deviations in growth hormone axis would play a part, since children with OI have been found to be unresponsive to stimulation with insulin-like growth factor I (Marini *et al.*, 1993), and about half of the patients with moderate OI had beneficial effects in a study with growth hormone therapy (Marini *et al.*, 2003).

In addition to bone, OI also affects other types of connective tissue. The laxity of ligaments lies behind the hypermobility of joints, which is a common symptom. It can lead to manifold orthopedic problems, pes planovalgus being particularly frequent (Mirzayan *et al.*, 2000). Other extraskkeletal features characteristic of OI include skin hyperlaxity, bruising and bleeding tendency. The overlap in the features of Ehlers-Danlos syndromes is evident. Patients with OI have an increased risk of valvular heart diseases, most frequently involving the aortic and mitral valves, and increased aortic diameter (Ashournia *et al.*, 2015). However, cardiovascular involvement is relatively rare in OI, with the prevalence of aortic valve regurgitation, the most common valvular abnormality, being 1.8%. It does not correlate with the severity of OI, either (Hortop *et al.*, 1986).

One distinctive feature of OI is bluish hue of the sclerae. Most of the people with OI have blue sclerae, but some will have white sclerae. The bluish hue may be a result from decreased scleral thickness, but it can also occur with normal thickness. In the latter case, it has been suggested to be a result of a different proteoglycan compositions, and therefore, different hydrations, reflecting wavelengths of blue color (Marini and Smith, 2015). Another distinctive feature of OI is Wormian bones, which are abnormal ossicles

that develop from accessory ossification centers within the cranium (Bellary *et al.*, 2013). They are frequently found in infants with moderate to severe OI. Both features have little clinical significance but they serve as diagnostic signs supporting the diagnosis of OI.

One of the most serious complications of OI, and occurring predominantly in patients with the more severe subtypes, are cranial base anomalies (Cheung *et al.*, 2011; Kovero *et al.*, 2006). These anomalies are divided into three categories: 1) platybasia meaning flattening of the skull base, 2) basilar impression where cranial base is lowered in relation to spine and the uppermost vertebral structures are located above the caudal border of the skull, and 3) basilar invagination where the uppermost vertebral structures protrude into the cranium through foramen magnum. These anomalies are separate but often coincide (Kovero *et al.*, 2006). Chiari I malformation is a frequent comorbidity, a prevalence up to 33-38% having been reported in patients with cranial base anomaly (Pindrik and Johnston, 2015). The anomalies may cause compression of the brain stem or spinal cord and their related neurovascular structures, leading to disturbed circulation of the cerebrospinal fluid, vascular compromise, as well as sensory and motor dysfunctions (Menezes, 2008), even ending in most severe cases to the patient's death (Sawin and Menezes, 1997). To prevent the most severe symptoms, neurosurgical treatment is in some extreme cases necessary (Hansen *et al.*, 2008; Sasaki-Adams *et al.*, 2008). Craniocervical anomalies, especially platybasia, can also be asymptomatic. Since platybasia usually occurs asymptotically, its clinical significance has been questioned. A baseline cephalometric examination with lateral skull radiograph of all OI patients before school age has been recommended as a screening method for cranial base anomalies (Storhaug, 2002). An individually adjusted plan for follow-up and treatment of those patients with abnormal findings is then warranted (Arponen *et al.*, 2012). MRI and CT are used for further evaluation of abnormal findings in lateral skull radiography, and MRI is the optimal modality for abnormalities of brain and spinal cord (Khandanpour *et al.*, 2012).

The most prominent dental aberration associated with OI is dysplastic dentin formation, in the literature, very often referred to as type I dentinogenesis imperfecta. The involvement of dentin is explained through type I collagen being the most important structural protein in dentin as well as in bone. Dentinal abnormality has been observed in 28 to 43% of OI

patients (Lukinmaa *et al.*, 1987; Lund *et al.*, 1998; Malmgren and Norgren, 2002; Schwartz and Tsipouras, 1984). The severity of dental findings is, however, rather a continuum, and hence milder aberration may remain underdiagnosed (Waltimo *et al.*, 1996). Dentinal abnormality is not associated strictly with any OI subtype, though the probability rises with the clinical severity of OI (Malmgren and Norgren, 2002). The color of affected teeth is typically yellow-brown or opalescent gray, but it may vary within the dentition, as well as between primary and permanent dentitions (O'Connell and Marini, 1999). The permanent dentition is almost always less affected. Although enamel is normal, it tends to fracture easily due to abnormal dentin, leading to significant attrition in a short time-period in some patients. Full coverage crowning after the full eruption of the primary dentition is often used at least in the primary molar area to maintain the appropriate vertical dimension of occlusion. In the permanent dentition, the dental care is usually more conservative. Radiographic signs of dentinal abnormality include bulbous shape of crowns, cervical constriction, short and thin roots and over time obliterating pulpal chambers. The diagnosis is usually based on clinical and radiological findings, although some OI patients with clinically and radiographically normal teeth may display histologically abnormal dentin (Waltimo *et al.*, 1996). In addition to structural dentin abnormality, OI patients also have an increased prevalence of hypodontia and malocclusions. The prevalence of hypodontia is reported to be as high as 20% (Lukinmaa *et al.*, 1987; Malmgren *et al.*, 2017). Typical malocclusion problems are class III malocclusion, anterior and posterior open bites, crossbites, and impacted teeth, and are more severe than in healthy individuals (Rizkallah *et al.*, 2013; Eimar *et al.*, 2016). The etiology of most of these malocclusion traits in OI resides in the vertical underdevelopment of the jaws (Waltimo-Siren *et al.*, 2005).

The majority of adult patients with OI have functional hearing loss. In the first two decades of the life, hearing loss is relatively infrequent and is mostly detected only as a subtle audiometric abnormality (Kuurila *et al.*, 2000). It exceeds functional stage in some patients in early adulthood, and up to 50% of the patients older than 50 years, report hearing loss, pathological audiometric findings being even more common (Kuurila *et al.*, 2000; Paterson *et al.*, 2001). The hearing loss related to OI is both conductive and sensorineural, and resembles that found in otosclerosis. Stapedectomy has good long term

results, but because of anatomic anomalies in the middle ear and tendency for profuse bleeding that are characteristic for OI, the operation is difficult (Kuurila *et al.*, 2004). There are no long-term results about the benefits of cochlear implants, though short-term results are promising (Streubel and Lustig, 2005).

2.3.4 Classification

The clinical severity of OI is a continuum. However, in planning the treatment and rehabilitation as well as assessing the prognosis, categorization of the patient is necessary. Therefore, through the history, patients have been categorized in different ways. At the beginning of the 20th century, the patients were divided into two groups of OI: congenital and tarda, the first one being more severe and the latter milder with fractures only occurring after birth (Chawla, 1964). This classification was, however, inevitably inadequate for the clinical management of the patients.

Thus, in 1979, Sillence and colleagues introduced a classification method for OI (Sillence *et al.*, 1979), which is still in use, though with some modifications. The classification divided OI patients into four groups according to clinical characteristics and pattern of inheritance. The classification does not take genetic background into account, and since knowledge about the genetic background of OI has increased, there has been debate on the deficiencies of the classification. At first, when mutations in new genes causing OI were described, new subtypes were added to the classification, representing these patients. The number of these genes, however, has grown rapidly, and this practice has been questioned. The latest nomenclature recommends dividing OI in five groups (Van Dijk and Sillence, 2014).

Type 1 OI comprises patients with mild disease and is the most common of the subtypes. The patients exhibit relatively mild bone fragility, and they do not have significant bone deformities or height deficit. Vertebral compression fractures are, however, typical and can lead to scoliosis, though rarely. Almost all the patients with type 1 OI have distinctly grey-blue sclerae. Type 2 OI is the severest form and it is lethal during the perinatal period. The fetuses experience multiple long-bone and rib fractures already in the uterus.

The latter one causes respiratory failure, which is the major cause of death after birth. Of those patients, surviving the neonatal period, the severest form of OI is type 3. These patients have significant bone deformities and very short stature and suffer multiple long-bone and vertebral fractures already prenatally or during the early stages of their life. Respiratory difficulties due to multiple rib fractures and severe kyphoscoliosis are the leading cause of death in this patient group as well. Sclerae are typically blue at birth but often become progressively whiter during life. The patients with moderate phenotype of OI are classified as type 4. These patients have moderately increased fracture incidence, mild to moderate bone deformities, and variably short stature. Sclerae are normally white, though some bluish hue may be seen at birth. This patient group is the most versatile, the severity typically varying even within families, some family members having considerably mild OI. The rarest subtype within this classification of OI is type 5. It is characterized by progressive calcification of the inter-osseous membranes in the forearms and legs, as well as by increased propensity to develop hyperplastic callus. Calcification of the inter-osseous membranes in forearms leads to restricted pronation and supination. Histomorphometric appearance of bone is characteristically coarse or mesh-like. The clinical severity is moderate and the sclerae normal. The causative genes for the OI types are listed in Table 2. (Bonafe *et al.*, 2015; Van Dijk FS and Sillence DO, 2014)

2.4 Bisphosphonates

2.4.1 History

Bisphosphonates (BPs) were first synthesized in 1865 in Germany and since then they have been known by chemistry (Menschutkin, 1865). They were used in industry as corrosion inhibitors or as complexing agents in the branches of textile, fertilizer, and oil industry. It was not until the 1960s when medicine became interested in BPs. Fleisch and colleagues had earlier found that inorganic pyrophosphate, contained in plasma and urine, inhibits *in vitro* formation and dissolution of calcium phosphate crystals (Fleisch and Bisaz, 1962). Since it also inhibited ectopic calcification *in vivo*, it was suggested that it

might act as a local physiological regulator of calcification and decalcification (Fleisch *et al.*, 1966). Because rapid hydrolysis precluded broader use of pyrophosphate as a therapeutic agent, Fleisch and colleagues turned to BPs that had similar physicochemical effect but resisted enzymatic hydrolysis. The first report about medical use of BPs was published in 1969 (Fleisch *et al.*, 1969).

2.4.2 Structure

BPs are structural analogues of pyrophosphates where oxygen connecting the two phosphates is substituted by a carbon atom. The P-C-P backbone accounts for the high affinity of BP to hydroxyapatite. The P-C-P structure has two lateral chains (R^1 and R^2) that allow great variability, and it is these chains that are modified to synthesize different BPs. The first generation of BPs used pharmacologically, such as etidronate and clodronate, did not contain nitrogen. It was then, however, discovered that positioning a nitrogen atom in the R^2 -chain increases the pharmaceutical potency of the molecule by 10 to 100 folds. To date, most of the BPs contain a nitrogen atom. The affinity of BP to hydroxyapatite can also be increased by adding a hydroxyl group (-OH) in the R^1 -chain. (Giger *et al.*, 2013)

2.4.3 Pharmacokinetics

BPs are to date administered either orally or intravenously. The absorption of the oral BP through the gastrointestinal canal is limited, the average bioavailability being only 0.3 to 7% (Ezra and Golomb, 2000). BPs chelate calcium in the gastrointestinal tract lowering even further the fraction of absorbed BP. In real life, food, drink, and cations decrease markedly the absorption, and BPs are hence recommended to be taken 30 minutes before breakfast (Ezra and Golomb, 2000). Approximately 50% of BP reaching the bloodstream is rapidly eliminated by the kidneys. The rest binds to the skeleton where it is not released from before the bone is resorbed. Therefore, their skeletal half-life is extremely long, up to several years (Giger *et al.*, 2013). Only the non-nitrogen-containing BPs are metabolized to cytotoxic ATP analogues, others remaining intact. The main route of elimination is via kidneys and only a fraction is eliminated through bile (Cremers and Papapoulos, 2011).

2.4.4 Mechanisms of action

The molecular mechanism of action of BPs remained long unknown but much has been learned recently. The two groups, nitrogen-containing and non-nitrogen-containing BPs affect osteoclasts through different pathways. When taken up by endocytosis into an osteoclast, nitrogen-containing BPs inhibit the mevalonate pathway of cholesterol synthesis, especially farnesyl pyrophosphate synthase, the main enzyme of the pathway. Hence, they prevent prenylation of small GTPases, leading to inhibition of the formation of the ruffled border, membrane trafficking, and transcytosis of degraded bone matrix. They can ultimately induce apoptosis which seems not to be the main mechanism of action, however. (Eghbali-Fatourehchi, 2014; Rogers *et al.*, 2011) The non-nitrogen-containing BPs are instead in osteoclasts incorporated metabolically into methylene-containing ATP analogues. The osteoclasts cannot degrade these cytotoxic ATP analogues, and they condensate and accumulate in the cytosol of the cell, leading to apoptosis (Eghbali-Fatourehchi, 2014). BPs have also been reported to inhibit apoptosis of osteoblasts and osteocytes at low concentrations (Plotkin *et al.*, 1999). These effects on osteoblasts and osteocytes are, however, of markedly less significance than the direct effects on osteoclasts. Different BPs with their wide range of potency to inhibit bone resorption are shown in Table 3.

Table 3: Characteristics of different bisphosphonates. Their relative potency to inhibit bone resorption *in vitro* is given, etidronate serving as a reference (Shaw and Bishop, 2005). Administration depicts available routes of administration.

	Nitrogen-containing	Administration	Relative potency
Etidronate	No	Oral	1
Clodronate	No	IV/Oral	10
Pamidronate	Yes	IV/Oral	200-500
Olpadronate	Yes	Oral	200-500
Ibandronate	Yes	IV/Oral	500-1,000
Aledronate	Yes	Oral	1,000-2,000
Risedronate	Yes	Oral	2,000
Zoledronic acid	Yes	IV	10,000

IV=intravenous

2.5 Medical therapies in children with OI

2.5.1 Bisphosphonates

The first case report concerning BP treatment in a pediatric patient with OI was published in 1987 by Devogelaer and colleagues (Devogelaer *et al.*, 1987). They reported notable clinical and radiological improvement in a 12-year-old female with OI after one year of oral pamidronate treatment. It took, however, until the late 1990s that larger observational studies about the benefits of BP treatments in pediatric patients with OI were published (Glorieux *et al.*, 1998), and BPs started to become more common as part of the treatment of patients with OI. Nowadays, the use of BPs is widespread in children with OI, especially in its moderate to severe forms. However, since large double-blind randomized placebo-controlled studies with intravenously administered BPs are still lacking, the benefits of BP treatment have been questioned. For instance, in USA, BP treatment is not an indicated therapy for OI.

A large number of observational studies have shown that during BP treatment children with OI experience a marked increase in BMD and bone mass (Glorieux *et al.*, 1998; Lindahl *et al.*, 2016; Rauch *et al.*, 2003). They also suggest a decrease in fracture incidence (Glorieux *et al.*, 1998; Lindahl *et al.*, 2016; Plotkin *et al.*, 2000) and an improved vertebral shape (Astrom *et al.*, 2007; Land *et al.*, 2006). Various aspects of the quality of life, such as bone pain, general well-being, and ability to perform in daily living, have also been shown to improve (Astrom and Soderhall, 2002; Land *et al.*, 2006). The dentinal dysplasia is unaffected since no osteoclasts exist in developing or mature dentin. The studies have mostly comprised patients with moderate to severe OI, and thus the benefits of the treatment in milder forms are still unclear. The first studies were performed using intravenous pamidronate, which is still the most widely used BP in children. Recently, more potent intravenous BPs, zoledronic acid and neridronate, have however been increasingly used, mostly because of their less frequent administration compared with pamidronate. The results with zoledronic acid and neridronate have been similar to those with intravenous pamidronate (Barros *et al.*, 2012; Gatti *et al.*, 2005). The results of the only double-blind randomized placebo-controlled study are shown in Table 4, and do not support the finding of observational studies in decreasing fracture incidence and bone

pain, or improvement in general well-being (Letocha *et al.*, 2005). Obstacles to larger randomized trials include, however, lack of interest among patients and their families to risk being randomized to placebo, and lack of interest among clinicians who have experienced the benefits of BP treatment among their patients.

In the last few years, double-blinded randomized placebo-controlled studies have demonstrated that also oral BPs have beneficial effects in children with OI. Oral risedronate and olpadronate increased BMD and reduced fracture risk (Bishop N *et al.*, 2013; Rauch *et al.*, 2009; Sakkars *et al.*, 2004). Oral alendronate was found to increase BMD in children with moderate to severe OI, but no significant decrease in fracture incidence was seen (Ward *et al.*, 2011) (Table 4). Traditionally, oral BPs are customary to use in adult populations. Currently, oral BPs are, however, also used with pediatric patients with milder forms of OI. Another patient population propitious for oral BPs is those children suffering needle phobia or refusing intravenous BP treatment for some other reason.

Histomorphometric and radiographic studies have shown that the main effect of BPs in growing patients with OI, i.e. the gain in bone mass, is due to increase in cortical thickness and trabecular number, while trabecular thickness and architecture remain unchanged (Rauch *et al.*, 2002; Apolinário *et al.*, 2016). Increase in cortical thickness resembles reduced osteoclastic activity in modeling; osteoblasts on the outer surface of cortex form new bone faster than osteoclasts on the inner surface resorb the older. The increase in trabecular number is instead due to a decrease in remodeling activity. The high mineralization stage characteristic of OI bone is found to remain unchanged (Weber *et al.*, 2006). The effect of BP treatment on intrinsic material properties of bone still remains unclear, studies with murine models and human patients reporting contradictory findings (Kashii *et al.*, 2008; Shahnazari *et al.*, 2010; Uveges *et al.*, 2009).

Table 4: Double-blind randomized placebo-controlled trials of bisphosphonate (BP) treatment in children with osteogenesis imperfecta (OI). (Bishop *et al.*, 2013; Letocha *et al.*, 2005; Rauch *et al.*, 2009; Sakkars *et al.*, 2004; Ward *et al.*, 2011). LS BMD, bone mineral density at lumbar spine.

Study	BP regimen	Treatment Time (months)	No. of Patients (BP/placebo)	OI Types (1/3/4)	LS BMD (BP/placebo)	Peripheral Fracture Rate	Bone Pain	Quality of Life
Sakker <i>et al.</i> 2004	olpadronate	24	16/18	13/9/12	Increased (+1.67 SD/+0.14 SD)	31% decrease	NA	No difference in self-care, mobility, or muscle strength
Letocha <i>et al.</i> 2005	pamidronate	12	9/9	0/9/9	Increased (+1.4 SD/no change)	Decreased in upper but not in lower extremities	No difference	No difference in mobility or muscle strength
Rauch <i>et al.</i> 2009	risedronate	24	13/13	26/0/0	Increased (+0.65 SD/-0.15 SD)	No difference	No difference	No difference in grip force
Ward <i>et al.</i> 2011	aledronate	24	109/30	32/39/54 (+14*)	Increased (+1.32 SD/+0.14 SD)	No difference	No difference	No difference in self-care, mobility, or grip force
Bishop <i>et al.</i> 2013	risedronate	12	94/49	121/5/17	Increased (+0.43 SD /-0.01 SD)	41% decrease	No difference	No difference in mobility

* patients with unknown OI type, NA=not analyzed

The most common side effect reported with intravenous BPs is the typical acute-phase reaction with influenza-like symptoms. The reaction is characterized with fever, malaise, nausea, myalgia, and sometimes diarrhea (Glorieux *et al.*, 1998; Hogler *et al.*, 2004). Approximately 85% of the patients experience at least some of these symptoms in the first 72 h after the first infusion. Reactions with the subsequent infusions are rare. Other common short-term side effects include transient hypocalcemia, hypophosphatemia, and rise in C-reactive protein, which rarely are symptomatic or of clinical significance. However, correction of possible preexisting vitamin D deficiency prior BP treatment, and supplementation of calcium before and after the first infusion are recommended (Bachrach and Ward, 2009; Hogler *et al.*, 2004). As more severe acute phase responses, a few cases of respiratory failure during pamidronate infusion cycle have been reported in children with OI (Munns *et al.*, 2004; Olson, 2014). The use of oral BPs has been associated in adults with chemical esophagitis and esophageal ulcerations. The understanding of proper administration, patient takes an adequate amount of water with medication and remains uprights for more than 30 minutes afterwards, have decreased these problems (Orozco and Maalouf, 2012). In children, the use oral BPs is not recommended for patients with risks for gastrointestinal disease, and for those receiving oral BPs, regular monitoring for gastrointestinal symptoms are suggested (Boyce *et al.*, 2013).

Since BPs remain in the body for years and experimental studies have shown them to cross the placenta, a concern has been that BPs would also effect developing fetus. Moreover, in animal models, they have been associated with skeletal anomalies in offspring (Patlas *et al.*, 1999). However, human reports concerning women exposed to BPs before conception or during pregnancy did not demonstrate serious adverse effects to fetuses. Since the experience is, however, scarce, it has been recommended that BP treatment should be discontinued 6 to 12 months before conception (Suresh *et al.*, 2014).

One of the adverse effects associated to BPs that has attracted much attention in adults is osteonecrosis of the jaw. It has usually occurred in older patients undergoing BP treatment, usually intravenously administered, for malignant disease or osteoporosis, and associated strongly with invasive dental procedures. The reducing effect on bone turnover with anti-angiogenic characteristics of BPs are believed to be reason for this unfavorable

and therapy-resistant process (Kumar and Sinha, 2014). Despite the large studies and general concern, no cases of osteonecrosis of the jaw have been reported in children with OI treated with BPs (Hennedige *et al.*, 2013). Nonetheless, it has been suggested that all children should have a dental check-up with required dental procedures prior to the onset of BP treatment (Bhatt *et al.*, 2014). Since bone resorption is crucial to tooth eruption, it has been suggested that BPs would delay eruption of teeth. Animal models support this hypothesis (Grier and Wise, 1998), but evidence supporting it in human still remains scarce (Kamoun-Goldrat *et al.*, 2008). Furthermore, studies with animal models also suggest BPs to have detrimental effect on orthodontic therapies by slowing down the attended tooth movement (Iglesias-Linares *et al.*, 2010). The clinical significance of these findings yet remains unclear.

BPs have been found to interfere with growth plates, causing radiographically seen dense transverse lines in the metaphysis in every infusion. These sclerotic lines consist of cancellous bone that contains calcified cartilage (Rauch *et al.*, 2004). BPs can also impair normal metaphyseal inwaisting, leading to club-shaped deformities and undertubulation in metaphyses, although this finding was seen in a patient exposed to doses exceeding markedly the recommended treatment doses (Whyte *et al.*, 2003; Whyte *et al.*, 2008).

Since BPs reduce remodeling activity of bone, a general concern has been that especially long-term use of BPs reduces it to a stage where microcracks can accumulate and lead to stress fractures. This phenomenon has been seen in adult patients with osteoporosis treated with BPs, especially after prolonged use (Gedmintas *et al.*, 2013). The typical location of these stress fractures is the subtrochanteric region of the femur, and the fractures are called atypical femoral fractures (AFF). Some case reports concerning AFFs in adult OI patients have been published (Etxebarria-Foronda and Carpintero, 2015; Holm *et al.*, 2014; Manolopoulos *et al.*, 2013; Meier *et al.*, 2012), but it is still unclear whether growing children have similar problems. Recently, a case report emerged, however, reporting a BP-treated teenager with OI who sustained recurrent femoral fractures with features of AFF (Vasanwala *et al.*, 2016). One larger study has also evaluated this aspect in pediatric OI patients and suggests a changing pattern in femoral fractures associated with BPs (Nicolaou *et al.*, 2012). Since remodeling is an important part of fracture healing as well,

it has been speculated whether BP treatment interferes with it. The studies concerning the topic suggest delayed bone healing after osteotomy but not after fracture in association with BP treatment (Munns *et al.*, 2004; Pizones *et al.*, 2005). A more recent follow-up study reported markedly reduced incidence of delayed osteotomy healing, which may have at least partially attributed to changes in BP infusion protocol (infusions were not given for four months after the operation) (Anam *et al.*, 2015). Whether BPs should be ceased, and for how long, after osteotomy or acute fracture remains unclear. Contrary to concerns about adynamic bone, related to prolonged BP treatment, the cessation of BP treatment during growth leads to lowered levels of metaphyseal bone mineral in subsequently formed bone and increased risk of fractures in the junction of the treated and untreated bone (Rauch *et al.*, 2006). The question whether “drug holidays”, a cessation of BP treatment, or maintenance treatment with either less frequent cycles of intravenous BPs or with oral BPs would be safer and more beneficial, is still unclear and needs further studies.

2.5.2 Other medical treatments

Another anti-resorptive therapy for OI is denosumab. It is an antibody against RANKL, a cytokine crucial to osteoclastogenesis and osteoclast function (Lacey *et al.*, 1998). At a histological level, denosumab and BPs have similar effects on growing bone (Wang *et al.*, 2014). Denosumab has established its role in treatment of postmenopausal osteoporosis and other skeletal disorders in adults, but the experiences in children with OI are scarce (Hoyer-Kuhn *et al.*, 2016; Semler *et al.*, 2012). The advantage of denosumab compared with BPs is its much shorter duration of action, allowing better control.

Therapies with bone-anabolic agents have attracted general interest, especially since genetic defects underlying OI primarily affect osteoblasts. Teriparatide (recombinant human PTH) has been shown to stimulate bone formation and have a beneficial effect on BMD in adult patients with mild OI. The effect was, however, less obvious in patients with moderate to severe OI (Orwoll *et al.*, 2014). There is concern about neoplastic risk in children related to teriparatide, and no evaluation of its use in children with OI has been published. Antibody-mediated sclerostin inhibitors are a new approach to stimulate bone formation by increasing osteoblast activity through inhibition of Wnt pathway. Similarly

to teriparatide in adults with OI, sclerostin-inhibiting therapies have shown more promise with mouse models for mild OI than in mouse models for severe OI (Roschger *et al.*, 2014; Sinder *et al.*, 2013). It has been suggested that the reason for the less impressive results of bone anabolic agents in more severe OI is due to the already increased bone formation rate, characteristic of these patients (Trejo and Rauch, 2016). In addition to specific bone anabolic agents, growth hormone stimulates osteoblasts as well. Findings of small studies suggest that growth hormone therapy has an increasing effect on BMD and linear growth in children with OI (Antoniazzi *et al.*, 1996; Marini *et al.*, 2003), but the results are, modest at the best. Furthermore, no data on its beneficial effect on final adult height or fracture incidence have been published.

Adequate intake of calcium and vitamin D is important for children with OI, especially since the serum 25-hydroxyvitamin D concentration is independently associated with BMD (Edourdo *et al.*, 2011). High-dose vitamin D (2000UI) did not, however, prove to be better to low-dose vitamin D (400UI) in increasing lumbar spine BMD (Plante *et al.*, 2016). Calcium and vitamin D-rich food and proper supplementations are hence recommended for children with OI (Chagas *et al.*, 2012; Harrington *et al.*, 2014).

3 Aims of the Study

BPs are commonly used in the treatment of pediatric patients with OI. Much of their impact on the bone of growing children remains unclear, and there are still many general concerns regarding especially their long-term effects. The aim of this thesis was to evaluate the benefits and long-term safety of BP treatment in children with OI, as well as assess the use of zoledronic acid instead of most commonly used intravenous BP pamidronate in the treatment of children with mild OI.

The following specific aims were set for the present study:

- 1 To assess the efficacy and short-term safety of zoledronic acid, an intravenous BP, in pediatric patients with OI.
- 2 To study the association of BP treatment with the morphology of the cranial base and craniocervical junction, as well as with the development of cranial base pathology in growing OI patients.
- 3 To study the association of BP treatment with the characteristics of femoral fractures in children with OI.
- 4 To study the association of BP treatment during childhood with the development and eruption of the permanent teeth, as well as resorption of the primary teeth in children with OI.

The hypotheses were:

- 1 Zoledronic acid is an effective treatment in children with OI.
- 2 BP treatment is associated with decreased risk to develop cranial base pathology in children with OI.
- 3 BP treatment is associated with changed characteristics of femoral fractures in children with OI.
- 4 BP treatment is associated with delayed dental development and eruption of permanent dentition in children with OI.

4 Materials and Methods

4.1 Patients and controls

The study population of all four studies comprised children and adolescents with diagnosed OI followed at the Metabolic Bone Clinic, Children's Hospital, Helsinki University Hospital, Finland. The clinic is responsible for coordination of patient care in moderate to severe OI, and for planning and follow-up of medical treatment of all pediatric OI patients in Finland. Currently, approximately 90% of the Finnish pediatric OI patients are or have been under follow-up at the hospital.

4.1.1 Study patients

Inclusion criteria for patients:

STUDY I (efficacy and safety of zoledronic acid): (i) Diagnosis of mild OI, (ii) treatment with intravenous zoledronic acid, (iii) a minimum treatment duration of 12 months, and (iv) no prior BP treatment.

STUDY II (BPs and cranial base pathology): (i) Diagnosis of OI, (ii) treatment with BP during growth, (iii) availability of radiographic evaluation of the cranial base during or after BP treatment, and (iv) age under 25.

STUDY III (BPs and femoral fractures): (i) Diagnosis of OI, and (ii) born between 1990 and 2012.

STUDY IV (BPs and dental development/tooth eruption): (i) Diagnosis of OI, (ii) age between 3 and 16 years, and (iii) treated with BPs for a minimum period of one year.

Study IV included patient recruitment. Altogether 39 patients fulfilled the inclusion criteria. These patients were contacted by mail and phone, and 17 patients consented to participation of clinical evaluation and radiography, the most common reasons for not participating being lack of time or interest. One of the consented patients was not able to follow the study protocol due to current health problems and was since excluded from the study group. In addition, six patients not taking part in the clinical examination were included into the study group using already available data.

Table 5. Basic demographics of the study patients in the four studies

	Number of patients	Sex (males/females)	Mean age (range), years	Mean height (range), SD	OI type (1/4/3)
Study I*	17	8/9	9.95 (1.5–16.8)	-0.9 (-2.0–0.0)	17/0/0
Study II**	39	22/17	11.6 (0–24.8)	-2.6	20/9/10
Study III***	127 [24]	66/61 [14/10]	7.7 (1.0–18.5)	-2.9 (-11.5–+1.0)	26/73/28 [11/6/7]
Study IV	22	13/9	10.4 (3.9–15.7)	NA	16/1/5

* at the baseline, ** for cross-sectional analysis, *** for individual fractures [] number of patients with fractures, NA= not addressed

4.1.2 Bisphosphonate treatment

The BP treatment of each patient in the Metabolic Bone Clinic is individualized based on clinical features and symptoms, including sustained fractures and BMD. For the intravenously administered BPs, the annual dose/kg is standardized, but the infusion protocol depends on the age and disease severity of the patient. Most of the patients receive intravenous pamidronate (9 mg/kg/year divided into 3 to 12 1-3-day infusion cycles per year, depending on age and disease severity) or zoledronic acid (0.1 mg/kg/year divided into 2 to 4 infusions per year). A few patients with mild OI receive orally administered risedronate (2.5 mg/day for patients weighing 10-30 kg, and 5 mg/day for patients weighing over 30 kg). A “drug holiday” of at least one year was kept after 2 to 3 years of BP treatment. The patients were monitored at least yearly, and BP was restarted if BMD deteriorated or pain or new fractures occurred.

The concept of cumulative dose was applied to compare various treatments with different lengths and different BPs. It was expressed per body weight (mg/kg), and coefficients were used due to three different BPs. These coefficients, introduced by Shaw and Bishop (Shaw and Bishop, 2005), are based on the relative potency of the molecules to *in vitro* inhibit bone resorption (Table 3). The low bioavailability of orally administered risedronate (0.63%) was also taken into account.

4.1.3 Control groups

To assess the effect of BP treatment, control groups were included in Studies II and IV. In Study II, the control group comprised historical data of 70 patients with OI, who were aged between 0 and 39 years and were not treated with BP. It was used only in the longitudinal analysis. In Study IV, 50 patients with OI, aged 3 to 16 years, comprised the control group of OI patients naïve to BPs. In addition, regarding one parameter, the resorption of deciduous teeth, no reference values were available and historical data of randomly chosen 50 healthy Finns (aged 5 to 14 years) served as normal reference to assess the effect of OI. Studies I and III did not include separate control groups.

4.2 Methods

4.2.1 Data collection

Studies I, II, and III were retrospective cohort studies. In these studies, the data was retrospectively collected from patient records. The noteworthy data included height and medication data (Studies I, II, and III), fracture history (Studies I and II), neurological status and symptoms (Study II). For all femoral fractures, data were also collected regarding potential presence of intramedullary roding and the trauma energy leading to fracture (Study III).

Study IV was a cross-sectional study. Altogether 16 consented patients went through the clinical examination, and a dental panoramic tomograph (DPT) was obtained if one was not available within 3 years (12 patients). In addition, DPTs taken within 3 years of six additional patients who did not take part in the clinical evaluation were included in the analysis. The presence or absence of dentinal abnormality was assessed using clinical and radiologic criteria. Patient records were used for medication data.

4.2.2 Biochemical measurements (Study I)

The effect of zoledronic acid on bone turnover was determined using biochemical markers. Plasma ALP and serum PINP were used as markers of bone formation, while serum ICTP and urine NTx represented bone resorption activity. These markers were analyzed at the baseline and then semi-annually during the treatment, taking the last sample 6 months after the last infusion. To assess the short-term safety of zoledronic acid, the analysis included blood and urine samples obtained at the onset of each infusion and 6 months after the last infusion for parameters of calcium homeostasis, liver and renal function, and blood cell count. In addition, plasma concentration of calcium was measured 2 h after every infusion, and that of phosphate and parathyroid hormone 2 days later, to evaluate the acute-phase effect of treatment on calcium homeostasis.

4.2.3 Densitometry (Study I)

BMC and bone area at the lumbar spine, femoral neck, and whole body were measured at the baseline and then annually with DXA to evaluate the changes in areal BMD (aBMD) (g/cm^2). Measurements were performed using Hologic Discovery A, using pediatric software version 12.4. All values were transformed into z-scores using age- and sex-specific reference data for the equipment. To also take the shape of lumbar vertebrae into account, additional lateral projection of lumbar spine was obtained with DXA scanner, and volumetric BMD (vBMD) (g/cm^3) values were automatically calculated. For vBMD, no pediatric reference values were available.

4.2.4 Vertebral assessment (Study I)

The effect of zoledronic acid on development and healing of vertebral fractures was evaluated using conventional postero-anterior and lateral radiographs of the thoracic and lumbar spine. The morphology of each lumbar and thoracic vertebra was individually assessed using a standardized pediatric scoring system (Makitie *et al.*, 2005), regarding 20% or greater reduction in vertebral height abnormal. The spinal fractures were classified as anterior wedge deformities if anterior and middle vertebral heights were decreased as compared with the adjacent normal vertebrae. If anterior, middle, and posterior vertebral heights were all decreased, the classification was compression deformity. 20 to 49% of reduction was considered mild and the reduction of 50% or more severe. The improvement or deterioration of spinal changes was determined by comparing the grades assigned to each vertebra at treatment baseline and after 2 years of treatment.

4.2.5 Measurements of cranial base anatomy (Study II)

Lateral skull radiographs (94) and midsagittal MR (2) images were used to perform cranial base measurements. The three independent measurements (anterior cranial base angle, D-M angle, and McRae's measure) were calculated using 6 cephalometric landmark points (Figure 3). The reliability and applicability of the method for screening of basilar pathology has previously been reported by Arponen and colleagues (Arponen *et al.*, 2008). Since dimensions of the cranial base measurements change during growth, normal values

of healthy ethnic Finns were used as reference (Arponen *et al.*, 2008; Arponen *et al.*, 2010; Arponen *et al.*, 2012).

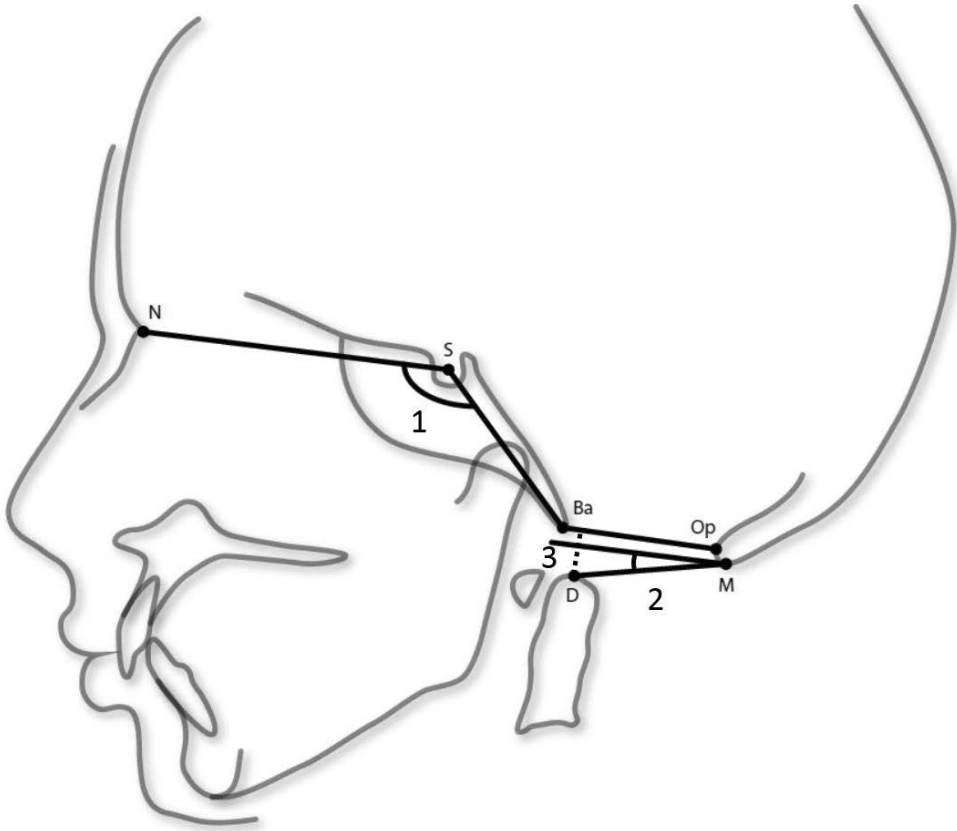


Figure 3: Cephalometric landmarks and reference lines. The illustration presents normal anatomy of cranial base structures. N = nasion, S = sella turcica, Ba = basion, Op = opisthion, D = dens (odontoid process), M = M-point. 1 = anterior cranial base angle, 2 = D-M angle, 3 = McRae's measure.

Cranial base measurements (Figure 3):

- 1) **Anterior cranial base angle:** The angle between the nasion-sella and basion-sella lines.
- 2) **D-M angle:** The angle between the line running through the lowest point of the occipital curve (M-point) parallel to the nasion-sella line and the line drawn from

the tip of the odontoid process to the lowest point of the occipital curve (normally negative).

- 3) **McRae's measure:** perpendicular distance of the tip of the odontoid process to the foramen magnum line, basion-opisthion (normally negative).

Following definitions and diagnostic criteria were used when evaluating cranial base anomalies:

- **Platybasia:** Anterior cranial base angle >2.5 SD above the average age-specific reference values.
- **Basilar impression:** D-M angle >2.5 SD above the average age-specific reference values.
- **Basilar invagination:** McRae's measure at zero or positive.

4.2.6 Characterization of femoral fractures (Study III)

The pattern of femoral fractures was assessed by characterizing the fractures according to the location of the fracture line within the femur, and fracture type by the shape of the fracture line. The assessment was performed using radiographs leading to fracture diagnosis. The location of fractures was categorized into four groups: 1) proximal fractures, including femoral neck fractures, intertrochanteric fractures, and slipped capital femoral epiphysis; 2) subtrochanteric fractures comprising the proximal third of the femoral shaft (diaphysis); 3) femoral shaft fractures comprising the mid and distal thirds of the femoral shaft; 4) distal fractures of femur comprising the fractures of distal metaphysis, physis, and epiphysis. Because of the rarity of other fracture types than transverse and oblique, the fracture type was categorized only into three groups: 1) transverse, 2) oblique, and 3) other fractures, which included fractures not fitting to these categories (such as spiral, comminuted, torus fractures, or slipped capital femoral epiphysis).

4.2.7 Methods to assess dental development (Study IV)

The developmental status of the permanent teeth, grade of resorption of the primary teeth and status of eruption of the permanent teeth were analyzed separately using DPTs.

The development of permanent teeth was assessed using the method introduced by Demirjian *et al.* (Demirjian *et al.*, 1973), which determines dental age by evaluating the radiographic appearance of seven permanent left mandibular teeth from the central incisor to the second molar. If one of the index teeth was missing or could not be evaluated for any other reason, the analysis was supplemented by the corresponding right-side tooth. The method is based on the progressive mineralization of each index tooth that is divided into eight stages (A-H). Stage A corresponds to the start of mineralization when only cusp tips are mineralized, and H to the end point of mineralization when the tooth is fully mineralized up to the root apex. To evaluate the difference from normal development, weighted norms for Finnish boys and girls were used (Chaillet *et al.*, 2004) and the assessed dental age, when differing from the chronological age by more than twelve months, was considered delayed or accelerated.

The assessment of the resorption stage of the primary teeth was performed based on the method introduced by Haavikko (Haavikko, 1973) with some modifications. In technique of the present study, the stage of resorption was divided into four categories according to the length of the root. The stages were: 1 = full root present, 2 = a quarter of the root resorbed, 3 = half of the root resorbed, and 4 = the root completely resorbed. Already exfoliated teeth were scored as 4. The resorption stage was assessed for each five primary teeth in the left mandibular quadrant, and the scores were summed up to form a resorption sum score, ranging from 5 to 20.

The eruption of the permanent teeth was assessed radiographically, and similarly limited to left mandibular quadrant. A tooth was considered emerged if it had pierced the alveolar bone cortex, whether or not it had pierced the oral mucosa. The number of erupted teeth was counted and compared with the published norms for timing of tooth eruption in healthy Finnish children (Nystrom *et al.*, 2001), considering values outside 5th and 95th percentile abnormal.

4.2.8 Statistical methods

With the continuous parameters, the differences between two groups were tested for significance using Student's t-test or Mann-Whitney U test, and differences between two related samples using Student's paired t-test or Wilcoxon signed-rank test, as appropriate. Analysis of variance was applied when comparing continuous variables in more than two groups. Categorical variables were tested with Chi-square test or Fisher's exact test, as appropriate. Correlations between two different variables were tested with Pearson's correlation analysis or Spearman's rank correlation. Generalized linear mixed model was used to analyze the association between patient characteristics and cranial base anomalies.

Statistical analysis was performed using StatView software (StatView 5.0.1; 1992-1998[©]; SAS Institute Inc., Cary, NC, USA) (Study I), the R-language (version 2.13.0, R Development Core Team, R: A Language and Environment for Statistical Computing (Vienna, Austria, 2008) (Study II), or SPSS software for Windows (versions 18 and 22; SPSS Inc., Chicago, IL, USA) (Studies II, III, and IV).

4.2.9 Ethical considerations

The studies were approved by the Research Ethics Committee, Children's Hospital, Helsinki University Hospital. Informed consent was obtained from all the participants and their parents/guardians of Studies II and IV, which included imaging for scientific purposes. The imaging was always also clinically justified and the radiation exposure was considerably low. Studies I and III were retrospective cohort studies and hence the patients were not contacted.

5 Results

5.1 Efficacy and safety of zoledronic acid

5.1.1 Efficacy

A total of 17 patients with type 1 OI and previously naïve to BPs had been treated with zoledronic acid in our clinic by 2009 (Table 5). Their median age at treatment onset was 10.1 years (range 1.5 to 16.8 years), and median treatment time 23 months (range 12 to 38 months). At the onset of treatment, these patients had a history of in total 73 peripheral fractures during their life time, making the fracture incidence in this cohort 0.52 per year. During the last 2 years preceding the treatment, the mean fracture incidence was 0.38 per year. After the treatment onset, the patients sustained altogether ten new peripheral fractures, making their mean fracture incidence during follow-up 0.28 per year. However, the decrease ($p=0.06$) in this cohort did not reach the level of statistical significance. When considering vertebral fractures, nine patients (53%) displayed compressed vertebral bodies in baseline spinal radiographs. Follow-up radiographs obtained after 24 months of treatment were available for nine patients. Of them, five had had compression fractures already at baseline and one additional patient manifested with a compression at follow-up. Of the five patients with compression fractures at baseline, two had new compressions at follow-up. However, three patients had fewer compressions than at baseline. At the onset of treatment, three patients suffered daily bone pain. After the treatment onset, two of them reported that pain disappeared. In one these two patients, the infusion interval was shortened to 3 months in order to prevent re-occurrence of pain.

At the treatment onset, the median lumbar spine aBMD z-score, for 14 patients whom it could be measured, was -2.0, and during the first treatment year, it improved to -1.0 ($p<0.001$). The improvement was more moderate during the second treatment year: for those ten patients who completed the 2 years of treatment, the median lumbar spine z-score improved from -2.3 to -1.2 ($p<0.001$) during the first treatment year, and from -1.2 to -0.7 ($p=0.01$) during the second treatment year. The aBMD values measured at whole body less head showed parallel but milder improvement (Figure 4). The biochemical

markers of both bone formation (plasma ALP and serum PINP) and bone resorption (urine NTx) showed a marked decrease in bone metabolism during the first six months of the treatment and stayed reasonably stable after it. During the first six months of the treatment, the average decrease in plasma ALP was 17% ($p<0.01$), in serum PINP 43% ($p<0.001$), and in urine NTx 46% ($p<0.01$). The second marker of bone resorption (serum ICTP) did not show a statistically significance difference during the treatment (Figure 5).

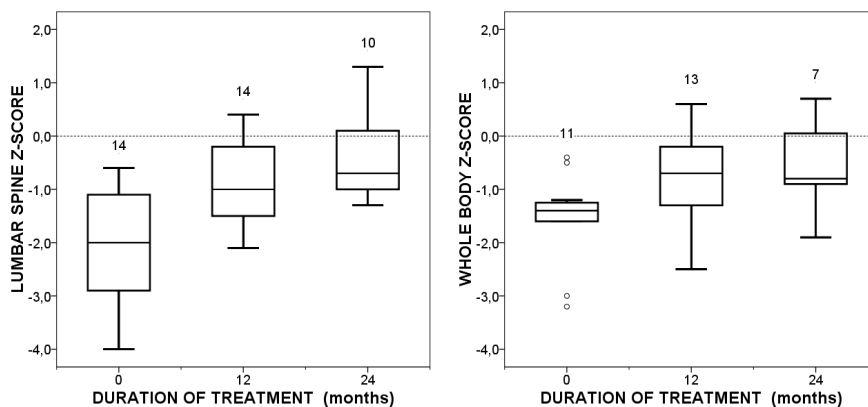


Figure 4. Areal bone mineral density z-score at lumbar spine and whole body measured at baseline and after 12 and 24 months of zoledronic acid treatment. The plot shows median, quartiles, and range. The numbers above plots refer to the number of observations at each time point.

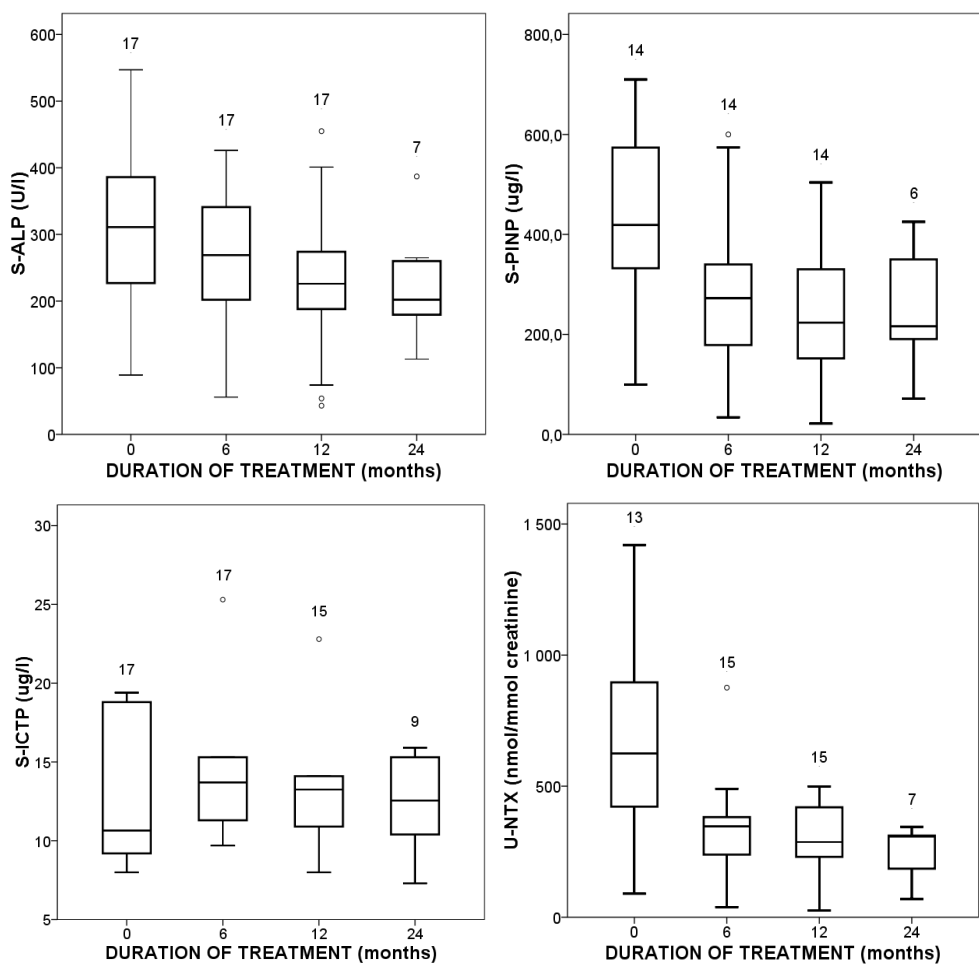


Figure 5: Biochemical markers of bone metabolism, serum alkaline phosphatase (S-ALP) and plasma procollagen type I N-terminal propeptide (P-PINP) referring to bone formation, and serum collagen type I C-terminal telopeptide (S-ICTP) and urine collagen type I N-terminal telopeptide (U-NTx) referring to bone resorption. The values are shown at baseline and after 6, 12, and 24 months after zoledronic acid treatment. The numbers above plots refer to the number of observations at each time point.

5.1.2 Safety

Overall, the treatment was well tolerated. The most common side effect was transient flu-like reaction after the first infusion that occurred in 11 out of 17 patients (65%), 5 of which reported bone pain as well. Only one patient had the same reaction after the second infusion. Plasma calcium values measured 2 days after the first infusion and the mean decrease after the first infusion was 9% ($p<0.001$). 12 patients had values below normal, of whom 2 had clinical symptoms, mainly fatigue, dizziness, nausea, and mild muscle tremor. These two patients were treated with intravenous calcium and the symptoms improved. Similar decrease in calcium levels was seen with subsequent infusions but without clinical symptoms (mean decrease 7-10%, $p<0.001$). The mean plasma phosphate decreased 41% ($p<0.001$) after the first infusion. After subsequent infusions, the decrease was between 20 and 32%. All changes were transient and normalized by the time of the next infusion.

5.2 Cranial base pathology

The study group comprised altogether 39 pediatric patients with OI representing a wide spectrum of severity (Table 5). Of these patients, 13 (33%) exhibited a cranial base anomaly, platybasia being the most prevalent diagnosis (28%). The prevalence of basilar impression was 18% and basilar invagination 15%. Logistic regression analysis suggested that a higher risk for basilar impression and basilar invagination correlated with the severity of OI (type 3, OR 22.04, 95% CI 1.26-386.28, $p=0.04$).

The cross-sectional analysis also suggested that a higher risk for basilar impression and basilar invagination correlated with older age at the onset of BP-treatment (OR 1.45, 95% CI 1.01-2.09, $p=0.04$) and short duration of the treatment (OR 0.28, 95% CI 0.087-0.09, $p=0.03$). No statistically significant association between cumulative BP dose and cranial base anomaly was detected.

Longitudinal radiographic/MRI data were available for 22 patients (56%), of which 8 patients exhibited at least one cranial base anomaly before the onset of BP treatment. Of them, four with only platybasia acquired a normal cranial base angle and did not develop

any other anomaly during the follow-up. Of those four with persistent cranial base anomalies, one acquired additional basilar impression and platybasia, and one additional basilar impression and invagination. All basilar impressions and invaginations persisted during the follow-up. Five patients not displaying any cranial base anomaly at the onset of BP treatment developed one during the follow-up period.

Kaplan-Meier analysis showed a statistically non-significant trend towards delayed development of cranial base anomalies in patients treated with BPs when compared with BP-naïve patients (basilar impression, log rank = 0.205 on 1 df, $p=0.651$; platybasia, log rank = 0.326 on df, $p=0.568$). None of the eight patients whose BP treatment was started before the age of 3 years developed later basilar impression or invagination during the follow-up.

5.4 The pattern of femoral fractures

Altogether, 149 femoral fractures had occurred to 25 OI patients during the study period. For 127 fractures that had occurred to 24 patients, data were sufficient and formed the final research material. The basic demographics of these 24 patients are illustrated in Table 5.

Among the 127 femoral fractures, the most common fracture site was mid or distal shaft (41%) followed by subtrochanteric and distal fractures, respectively. Regarding the fracture type, transverse fractures comprised approximately two thirds of all fractures. The remaining third were mostly oblique by configuration, other fracture types being rare (7%) (Figure 6). Analysis of the femoral fractures in the various OI subtypes revealed some statistically significant differences in fracture site: Distal fractures were more common in the more severe types 3 and 4 OI compared to the milder type 1 OI, whereas fractures of the mid or distal femoral shaft were more common in type 1 OI than in types 3 and 4 (Table 6).

Regarding BP treatment, 50% of the fractures had occurred in patients naïve to BPs, 35% during the BP treatment, and 20% during “drug holiday”. The distribution of fracture site

and fracture type was similar in all above mentioned groups, and no statistically significant associations could be found between BP treatment and the pattern of femoral fractures, either (Table 6).

Table 6. Distribution of site and type of femoral fractures in the three osteogenesis imperfecta (OI) subtypes and the three treatment groups

OI type		Bisphosphonate treatment								
		Type 1	Type 3	Type 4	p-value	Naïve	Ongoing	Discontinued	p-value	Total
Number of fractures		26	28	73		63	44	20		127
Fracture location					0.02				0.78	
	proximal	3 (12%)	2 (7%)	2 (3%)	0.13	3 (5%)	4 (9%)	0 (0%)	0.44	7 (6%)
	subtrochanteric	7 (27%)	10 (36%)	25 (34%)	0.75	20 (32%)	14 (32%)	8 (40%)	0.77	42 (33%)
	mid or distal shaft	16 (62%)	7 (25%)	29 (40%)	0.02	28 (44%)	17 (39%)	7 (35%)	0.70	52 (41%)
	distal	0 (0%)	9 (32%)	17 (23%)	0.004	12 (19%)	9 (21%)	5 (25%)	0.85	26 (20%)
Fracture type					0.11				0.35	
	transverse	12 (46%)	18 (64%)	53 (73%)	0.051	42 (67%)	26 (59%)	15 (75%)	0.44	83 (65%)
	oblique	10 (38%)	9 (32%)	16 (20%)	0.22	18 (29%)	12 (27%)	5 (25%)	0.95	35 (28%)
	other	4 (15%)	1 (4%)	4 (5%)	0.23	3 (5%)	6 (14%)	0 (0%)	0.11	9 (7%)

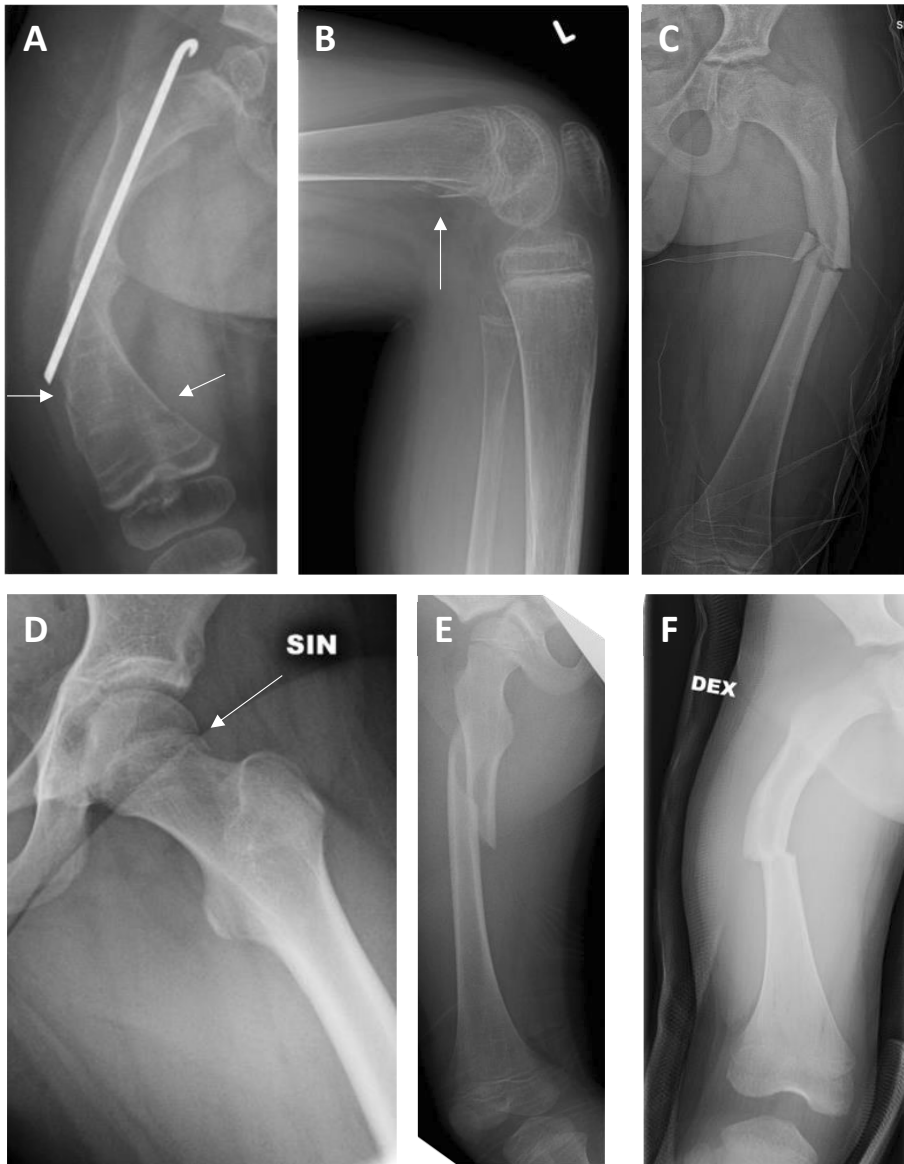


Figure 6: Examples of different fracture types. (A) An oblique fracture of the distal femur, shown by the white arrows, occurred in a 3.3-year-old girl with an ongoing bisphosphonate (BP) treatment. (B) A spiral fracture of the distal femur, shown by the white arrow, occurred in a 10.0-year-old boy naïve to BPs. (C) A comminuted fracture of the femoral shaft occurred in a 10.1-year-old boy with an ongoing BP treatment. (D) An epiphyseolysis of the proximal femur, shown by the arrow, occurred in a 12.8-year-old girl with an ongoing BP treatment. (E) A spiral fracture in the subtrochanteric region of the femur occurred in a 7.0-year-old boy with an ongoing BP treatment. (F) A transverse fracture of the femoral shaft occurred in a 7.0-year-old boy naïve to BPs.

5.3 Dental development

A total of 22 patients treated with BP:s comprised the final study group. Their dental development, in terms of radiologically assessed developmental stage of the permanent teeth, resorption of the deciduous teeth, and number of erupted permanent teeth, displayed age-appropriate stage when compared with healthy Finnish children ($p=0.23$, $p=0.23$, and $p=0.80$, respectively). When the patients were divided into two groups according to the presence dental abnormality, the development was still age-appropriate (Table 7).

Table 7: The difference between dental and chronological age in children with osteogenesis imperfecta, subgrouped by bisphosphonate treatment (BP) and dental abnormality (DI). Results are given as mean (range).

	n	Difference between dental and chronological ages (y)	<i>p-value</i>	Difference between number of erupted teeth and reference values	<i>p-value</i>
BP+/DI-	17	+0.37 (-1.49 - +3.89)	0.47	-0.18 (-2 - +1)	0.32
BP+/DI+	5	+0.43 (-1.17 - +1.46)	0.35	+0.80 (0 - +2)	0.10
Total/BP+	22	+0.39 (-1.49 - +3.89)	0.23	+0.05 (-2 - +2)	0.80
BP-/DI-	31	+0.58 (-2.03 - +3.77)	<0.01	+0.29 (-3 - +3)	0.19
BP-/DI+	19	+0.93 (-1.43 - +3.77)	<0.01	+0.42 (-1 - +2)	0.03
Total/BP-		+0.71 (-2.03 - +3.77)	<0.001	+0.31 (-3 - +3)	0.03
Total/DI-	48	+0.51 (-2.03 - +3.89)	0.01	+0.13 (-3 - +3)	0.39
Total/DI+	24	+0.82 (-1.43 - +3.77)	0.04	+0.50 (-1 - +2)	<0.01

When OI patients naïve to BPs were compared to healthy individuals, they were advanced in terms of development of permanent teeth and number of erupted permanent teeth ($p<0.001$ and $p=0.03$, respectively). In the subgroup of patients with dental abnormality, the difference seemed to be even more evident (Table 7). In terms of resorption of the deciduous teeth, they did not differ statistically significantly from Finnish norms.

When the two BP groups were compared with each other, OI patients naïve to BPs were found to be advanced in terms of resorption of the deciduous teeth when age was used as covariate ($p=0.04$) (Figure 7). In BP-treated OI patients, as in healthy controls, root resorption in the deciduous tooth never began until the crown of the underlying permanent tooth was fully complete, whereas in 27% of the OI patients naïve to BPs, the resorption

started earlier. The difference in the developmental stage of the permanent teeth and the number of erupted permanent teeth was statistically non-significant ($p=0.21$ and $p=0.23$, respectively).

Neither treatment time nor relative cumulative dose correlated with the rate of dental development, resorption of primary teeth or eruption of permanent teeth ($p<0.05$).

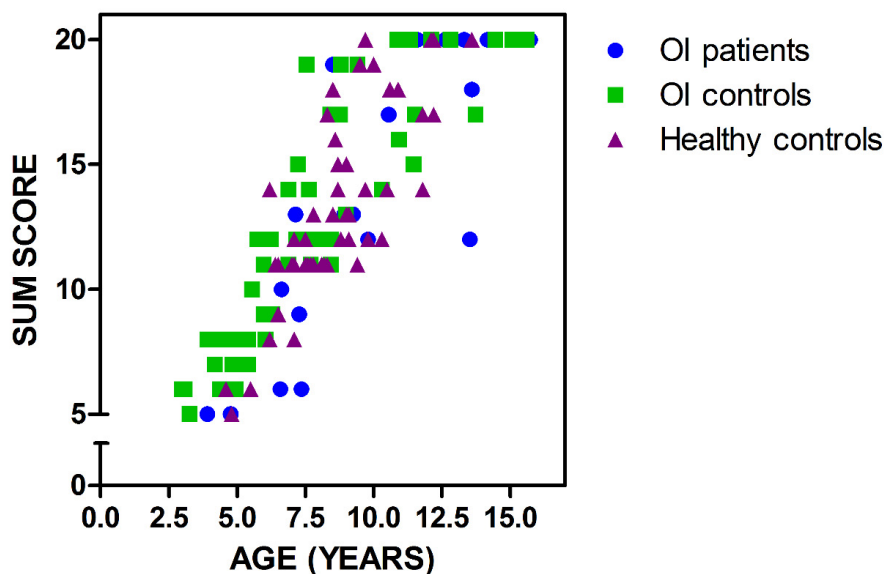


Figure 7: Correlation between age and resorption sum score. The resorption sum score was statistically significantly different between the three groups ($p=0.04$). The difference was statistically significant between osteogenesis imperfecta (OI) patients naïve to bisphosphonates (BPs) and those treated with BPs ($p=0.04$), but not when either group were compared to healthy controls ($p=0.23$ and $p=0.62$, respectively).

6 Discussion

6.1 Zoledronic acid treatment in children with mild OI

Traditionally, intravenous pamidronate has been the most commonly used BP in the medical treatment of children with OI. The superior potency and longer-lasting efficacy in suppressing bone resorption give zoledronic acid, compared with pamidronate, however, an advantage in terms of infusion protocol. In the clinic where the study was performed, the standard protocol of administration of zoledronic acid is one infusion over 45 min once every 6 months, which, compared with pamidronate given over 4 h in 3 successive days once every 4 months, makes it much more convenient to the families.

In the present study, children with mild OI showed a marked improvement in aBMD during the first year of zoledronic acid treatment. During the second year of treatment, the improvement was approximately halved. The improvement did not reach the level of improvement reported in other studies on pamidronate (Arikoski *et al.*, 2004; Glorieux *et al.*, 1998; Rauch *et al.*, 2003). However, the pretreatment BMC has been shown to negatively correlate with the increase in BMC during treatment (Rauch *et al.*, 2003). Previous studies on pamidronate have predominantly included patients with moderate to severe OI, and thus, the explanation for the different levels of improvement could reside in the baseline. Furthermore, the improvement in BMD was slightly more favorable in the present study as compared with the findings of a study on pamidronate comprising only patients with mild OI (Zacharin and Kanumakala, 2004). Similar findings were later reported by Barros and colleagues in an open-label, prospective, and randomized clinical trial, comparing 1-year treatments with zoledronic acid and pamidronate (Barros *et al.*, 2012).

The primary clinical outcome of BP treatment is fracture reduction. The present study suggested a decreasing trend in the fracture incidence during the treatment, but it was, however, statistically non-significant. Yet, it should be noted that since the susceptibility to suffer fractures varies considerably during the childhood in OI, the comparison to fracture history alone is insufficient for drawing definite conclusions. Three out of five

patients with vertebral compression fractures showed recovery during the treatment. On the other hand, new vertebral compressions were also diagnosed after 2 years of treatment, one occurring in a patient with no former history of compressed vertebrae. The radiographic evaluation of vertebral fractures in patients with OI is, however, challenging due to low BMD. Since some of these compressions were asymptomatic, it is possible that those compressions only became radiographically more visible during the treatment. A similar pattern of progress was seen in other the studies on pamidronate (Astrom *et al.*, 2007; Land *et al.*, 2006).

Zoledronic acid treatment was overall well tolerated, and none of the patients discontinued the treatment because of side effects. The most common side effect was the “acute-phase reaction” with fever and bone pain, characteristic of intravenous BPs. The reaction appeared only with the first infusion in all except one patient and was controlled with standard doses of acetaminophen. As with pamidronate treatment, significant changes in mineral homeostasis were detected after infusion but all these changes were transient and normalized by the subsequent infusion (Glorieux *et al.*, 1998). Two patients suffered clinically symptomatic hypocalcemia, however, and required treatment with intravenous calcium, which has not been reported with pamidronate. These findings underline the importance of adequate calcium and vitamin D supplementation before and during zoledronic acid treatment.

6.2 Long-term effects of BP therapy in children with OI

6.2.1 Cranial base pathology

Cranial base pathology is one of the most severe complications in OI, being in severe cases even life-threatening. Its prevalence increases hand in hand with the severity of OI (Cheung *et al.*, 2011; Sillence, 1994). In the perinatally lethal type 2 OI, it is, however, not seen (Sawin and Menezes, 1997). The pathogenesis of basilar impression and invagination in OI is believed to involve a combination of bone fragility and malleability, as well as ligamentous hyperlaxity, which decreases the ability of the cranial base to resist the compressing forces caused by the brain in an upright position. As a consequence, the

cervical spine migrates upwards in relation to the cranium. This theory is supported by the findings reported by Arponen and colleagues that basilar impression and invagination are detected only after the age of 2 years (Arponen *et al.*, 2012). The observations in the present study were also consistent with this.

In the cross-sectional analysis in the present study, the prevalence of cranial base pathology in the BP-treated children with OI was similar to the one found in a previous study on children with OI naïve to BPs, representing comparable distribution of clinical severity (Arponen *et al.*, 2012). In the longitudinal analysis, a non-significant trend towards delayed progression of cranial base pathology was also seen in the Kaplan-Meier curves in patients treated with BPs compared to the historical control group naïve to BPs. It is obvious that hyperlaxity of ligamentous structures plays a prominent role in the pathogenesis of cranial base anomalies in OI. It should be noted that BP treatment does not affect these structures because of the absence of hydroxyapatite. Also, the observation that none of the eight patients in whom the BP treatment was started before the age of 3 years developed basilar impression or invagination indicates that the early onset of BP treatment may have a favorable effect on the development of cranial base pathology.

Platybasia, a flat skull base, may instead be present already at birth. Since it often occurs asymptotically, its clinical significance has been disputed (Botelho and Ferreira, 2013). However, platybasia has been associated with basilar impression and invagination (Botelho and Ferreira, 2013; Kovero *et al.*, 2006), as well as suggested to predispose the development of Chiari type I malformation (Fernandes *et al.*, 2013). In platybasia, the clivus is oriented more horizontally than normally, which can be expected to co-exist with a more horizontally oriented and curved brainstem. This abnormal orientation is suggested to predispose to compression of the vertebral brainstem and tonsillar herniation. The longitudinal analysis in the present study indicated a recovery of platybasia during BP treatment in four patients with previous diagnosis, whereas in the previous study on patients predominantly naïve to BPs, platybasia persisted in all five patients originally diagnosed with it (Arponen *et al.*, 2012), indicating potential recovering effect of BP treatment on platybasia. However, platybasia has previously found to be less prevalent in

older OI patients (Kovero *et al.*, 2006), indicating potential naturally occurring recovery of platybasia.

Overall, the preventing effect of BP treatment on the development of cranial base pathology remains unclear, and further studies with larger patient cohorts and longer follow-up time are required.

6.2.2 The pattern of femoral fractures

In the last few years, atypical femoral fractures (AFFs) have been a hot topic in the treatment of osteoporotic adults. A meta-analysis of several large population-based studies suggests an increased risk of AFF in adult patient with osteoporosis treated with BPs (Gedmintas *et al.*, 2013). It should be noted, however, that the authors also state that the increased incidence of AFFs is likely to be outweighed by the overall reduction in the fracture risk associated with BPs. The same concern has recently received increasing attention in the treatment of children with OI, especially after Nicolaou and colleagues published their study, showing a remarkable shift towards subtrochanteric localization of femoral fractures associated to prolonged use of BPs (Nicolaou *et al.*, 2012).

AFFs are femoral fractures characterized by location in the shaft, a substantially transverse and minimally comminuted configuration, and flaring of the lateral cortex at the fracture site, which is detectable already before the fracture. According to the definition, AFF should also occur as a consequence of minimal or no trauma (Shane *et al.*, 2014). Many of these features are, however, also typical of fractures occurring in children with OI, even without exposure to BPs. In the present study, up to 76% of femoral fractures were located in the femoral shaft, and 67% represented a transverse configuration. Therefore, caution should be exercised before defining a fracture as “atypical” in OI. The pathogenesis underlying the brittleness of bone in children with OI differs markedly from the one in postmenopausal osteoporosis. In addition to impaired collagen type I protein, the bone matrix is in fact hypermineralized in OI in contrast to hypomineralization, characteristic of postmenopausal osteoporosis (Traub *et al.*, 1994). In addition to remodeling, the growing bone of children is, contrary to adults, abundant of modeling as well. Indeed, the ability of

BPs to increase bone mass in children with OI is thought to be mainly due to their inhibitive effect on osteoclasts in modeling, leading to increased cortical thickness (Rauch *et al.*, 2002) and reduced cortical porosity (Shapiro *et al.*, 2003).

In the present study, no difference in the characteristics of femoral fractures was found in terms of location and configuration related to BP treatment. Instead, the severity of the disease appeared to affect the pattern of fracture, location in the distal femur and transverse configuration being more common among the fractures occurred to patients with moderate to severe OI. Low bone mass and impaired material property of bone to resist bending forces in OI serve as a good explanation for the transverse configuration. The transverse configuration has, indeed, been found to be a frequent feature in long-bone fractures in OI, compared with healthy children (Dent and Paterson, 1991). The reason underlying the higher propensity to suffer distal femoral fracture is less clear. A previous finding that distal fractures also occur more frequently in children with disuse-associated osteoporosis (Lee and Lyne, 1990) suggests that disuse could be a contributor to the fracture risk, especially in more severe OI.

Previous reports concerning the effects of BP exposure on the bone material properties are contradictory. Some studies with murine models have suggested that BPs in fact decrease in the material strength of bone, leading to even more brittle bones (Kashii *et al.*, 2008; Uveges *et al.*, 2009). These findings were made after 12-week treatment, corresponding to a treatment from toddler to young adulthood. Human studies have, however, suggested that up to 5 years of treatment with pamidronate does not compromise these properties in children with OI (Rauch *et al.*, 2006; Weber *et al.*, 2006). The difference in the treatment length seems to be a logical explanation for the contradiction of the results in these studies. The treatment length was, in addition to the smaller BP doses, a major difference between the present study and the study by Nicolaou and colleagues as well (Nicolaou *et al.*, 2012). The average length of BP treatment preceding fractures was 6.5 years without standard “drug holidays”, whereas in the present study, the total duration was on average 4.1 years divided into 1-3 years of treatment periods. Shorter treatments and use of standard “drug holidays” may have had a protective impact on bone against the adynamic effects of BPs.

6.2.3 The dental development

The development and eruption of teeth requires resorption of the surrounding alveolar bone, and in case of permanent dentition other than molars, resorption of the roots of the primary teeth. As anti-resorptive agents, BPs have been speculated to compromise this process, delaying tooth eruption. The phenomenon has been demonstrated in murine models (Bradaschia-Correa *et al.*, 2007; Grier and Wise, 1998; Hiraga *et al.*, 2010). There is, however, great difference between rodent and human dentitions. Rodents have for example only one dentition, and their incisors are in a state of continuous eruption. The molecular mechanisms of these continuously erupting incisors and teeth of limited eruption have been found to differ markedly (Wise and King, 2008). Hence, the findings made in murine models cannot be directly applied to humans.

In humans, the evidence of delayed tooth eruption associated with BP exposure is scarce. Only one study considering the effect of BP treatment on tooth eruption has been published, by Kamoun-Goldrat and colleagues (Kamoun-Goldrat *et al.*, 2008). In their study, they observed a reduced number of clinically erupted teeth in BP-treated children with OI compared with matched healthy controls. In the present study, a delaying effect was found as well, which did not, however, cause abnormal eruption of permanent dentition since the dental development was advanced in OI patients naïve to BPs. One factor contributing to the contradictory findings may be the lack of radiographic data in the study by Kamoun-Goldrat and colleagues, which may have led to overestimation of the impact of BPs since the number of emerged permanent teeth was not corrected for congenitally missing and impacted teeth. The prevalence of hypodontia is approximately 20% in OI (Lukinmaa *et al.*, 1987; Malmgren *et al.*, 2017). The present study showed BPs to have a delaying effect on the resorption of the primary tooth roots as well. Since rodents have only primary dentition, it has not been possible to study this crucial part of eruption of most permanent teeth with murine models, and the finding is completely new, although not unexpected.

The finding that dental development is advanced in terms of dental age and eruption of the permanent teeth in OI, is new. Previously, there have been very few studies concerning dental development in OI. In one of the few, O'Connel and Marini reported an increased

variation in the timing of dental development among 27 children with moderate to severe OI – in 5 of them, the dental age was advanced and in 4 delayed by more than 12 months (O'Connell and Marini, 1999). However, this seemed to be of little clinical consequence. Malmgren and Norgren reported that 1 out of 68 children with OI represented delayed tooth eruption, and the only one deviation concerned eruption of primary teeth (Malmgren and Norgren, 2002). At tissue level, the finding of accelerated dental development in OI appears to be logical since the acceleration of osteoclastic and osteoblastic activity is characteristic of OI (Rauch *et al.*, 2000).

Overall, according to the findings of the present study, BP treatment by delaying seemed to normalize the timing of the development of permanent dentition inherently advanced in OI. Yet, it is worth noting that the delaying effect of BPs may be detrimental in children with other medical indications to BP treatment. Future studies are required to show if such an effect really exists and is of clinical significance.

6.3 Limitations of the study material

Besides the fact that the present study was mainly retrospective, several other limitations were identified during this study as well, primarily related to the size and heterogeneity of the study cohorts due to the rarity of OI. Regarding the long-term effects of BP treatment, the patient population comprised children treated with three different BPs, zoledronic acid, pamidronate, and risedronate, and in some patients two different BPs were even used in their treatment at different time points. Therefore, no statistical comparison between different BPs was possible, and coefficients were used when evaluating the cumulative dose response. Since BPs have established their role in the treatment of children with OI, patients naïve to BPs, especially with moderate to severe OI, are scarce. Historical data was therefore used in the evaluation of the impact of BP treatment. This can potentially introduce some bias to the results due to differences in the clinical management other than medical treatment. In the longitudinal analysis in Study II, and in Study IV, historical control groups with OI were used. In Study III, the control group comprised the fractures occurred in patients naïve to BPs. Regarding Study IV, the limited size of the control

group did not enable a proper age- and OI type-matching. The impact of age was minimized using reference values when available, and using age as covariate only with the resorption of the roots of the primary teeth. In Study II, age-matching was not required since the control group was used only in the longitudinal analysis. The study I did not include a control group at all, and it must be recognized that inclusion of a placebo or pamidronate group would have strengthened the data analyses. In Study III, the study material comprised fractures, which were divided into three groups according to status of BP treatment at the time of the trauma. Multiple fractures occurred in same patients were included, which may cause some bias. However, the use of possibly interrelated fractures should overestimate the impact of BP treatment and hence this does not challenge the results. As previously mentioned, the patient population can be estimated to comprise approximately 90% of the children with OI born during the study period. Therefore, to acquire larger and more homogenous patient populations, international co-operation is required in the future.

The data of the present study was collected during a time when “drug holiday” after 2-3 years of BP treatment was a common clinical practice in Finland. Hence, it is worth noting that the results concerning long-term effects of BP treatment should be generalized only to BP treatments carried out in a similar protocol. Currently, several centers worldwide use BPs for much longer periods or apply maintenance treatment with low dose BPs instead of “drug holiday”. In these situations, the adynamic bone effect of BP treatment may be more prominent.

6.4 Future perspective

Although three decades have passed since the first reports of the use of BP therapy in children with OI, and hundreds of studies concerning its efficacy and safety have been published ever since, much still remains unclear. This is especially true with the optimal treatment protocol. Whether treatment should be paused at 2-3 years, after the maximal benefits on bone mass and density have been obtained, and restarted individually according to need, or whether maintenance treatment with a lower dose of intravenous or oral BP should be applied, remains an open question. Since maintenance treatment has

nowadays been applied more and more frequently, also here in Finland, it would be interesting to compare these two treatment protocols concerning for instance potential fractures occurring in the junction of the treated and untreated bone, and manifestations of adynamic bone effects of BP treatment. Furthermore, since the knowledge of the impact of BP treatment on fracture healing is still scarce and considering the available data on 127 femoral fractures, it seems logical also to evaluate the healing of these fractures and assess the impact of BP treatment on it.

7 Conclusion

The purpose of this study was to evaluate benefits and long-term safety of BP therapy in the treatment of children with OI, and to assess the use of zoledronic acid instead of the most commonly used intravenous BP pamidronate in the treatment of children with mild OI. Based on the findings of the study, the following conclusions are presented:

- I** Intravenous zoledronic acid can be regarded as effective and safe treatment in children with mild OI.
- II** Cranial base pathology may develop despite BP treatment. Treatment started in infancy may, however, have a delaying effect on the development of cranial base anomalies, supporting an early initiation of the treatment.
- III** No evidence of changing pattern in femoral fractures related to BP exposure was found in children with OI. Instead, the characteristics of these fractures seemed to reflect the severity of OI.
- IV** BP treatment delays the development and eruption of permanent dentition in patients with OI. Since the development and eruption of permanent dentition is according to our findings inherently accelerated in children with OI, the overall delay due to BP exposure in therapeutical doses is likely to normalize the timing of permanent dentition to a level comparable to unaffected children.

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